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MORPHOLOGY OF SPOROTRICHUM SCHENCKII AND HISTOPLASMA CAPSULATUM IN TISSUE

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AND
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HISTOPLASMOSIS and sporotrichosis may be regarded histopathologically as cytomycoses, since the causative organisms occur principally within macrophages.

A peculiarity of the organism causing these two diseases is their dimorphism; i. e., the form seen in tissue differs in appearance from that in culture. The yeastlike budding forms observed in the animal host stand in contrast to the moldlike filamentous growth observed on Sabouraud's medium at room temperature.

Histoplasma capsulatum in tissue is conventionally described as an organism which is surrounded by a clear capsule.[1] This is implied by the specific name of the organism. The cells of H. capsulatum are 2 to 4 microns in diameter and are crowded within the macrophages of the reticuloendothelial system. In preparations stained with hematoxylin and eosin the organism is seen to be composed of a basophilic, oval or crescentic mass, usually not more than a micron or two in diameter, centrally or eccentrically placed with respect to a clear halo (the so-called "capsule") which surrounds the basophilic mass. The halo is even more evident in Giemsa-stained preparations. The tissue phase of the organism is often referred to as the "yeast phase" because of the capacity of the fungous cells to multiply by budding. When the organism is cultured at 37 C. on mediums containing blood, the yeastlike tissue forms of the fungus are reproduced but these are noncapsulated.

Recently, Neill and his associates[2] have recorded their observations on the "capsule" of Sporotrichum schenckii in animal tissue. These workers demonstrated a *Quellung* reaction, or swelling, of the capsule when organisms collected from infected mouse tissue were mixed with homologous antiserum. According to this work, the organisms making up the tissue or yeast phase are encapsulated. Although

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We wish to acknowledge the assistance of Dr. Thomas F. Anderson and Mr. Carl Oster of the Johnson Foundation, Hospital of the University of Pennsylvania, in the preparation of the electron photomicrographs.

1. Skinner, C. E.; Emmons, C. W., and Tsuchiya, H. M.: Henrici's Molds, Yeasts and Actinomycetes: A Handbook for Students of Bacteriology, ed. 2, New York, John Wiley & Sons, Inc., 1947.

2. Neill, J. M.; Castillo, C. G.; Smith, R. H., and Kapros, C. E.: J. Exper. Med. **89**:93, 1949.

the literature contains little reference to the capsule of S. schenckii in animal tissue, the published photographs of invaded tissue sometimes quite clearly reveal halos or clear zones around the organisms.[3] These zones certainly suggest capsules. As with H. capsulatum, incubation of this organism at 37 C. on blood medium permits the development of the budding, yeastlike phase seen in tissue. However, the cultured yeast phase organisms are not encapsulated, being similar in this respect to H. capsulatum.

The object of the present report is to describe the structure of the tissue phases of H. capsulatum and S. schenckii in the animal host with particular reference to the question of whether or not these organisms are encapsulated.

METHODS

Three techniques were employed in the studies of the tissue phases of these organisms.

1. Histologic study of biopsy specimens stained in various manners as described below.
2. Electron microscopy.
3. The *Quellung* reaction.

The experimental infections were established in chick embryos and mice. The chorioallantoic membrane of the chick embryo was shown by Moore [4] to be an excellent substrate for the growth of pathogenic fungi. More than a hundred 9-day old embryos were inoculated with each organism by depositing 0.1 ml. of a dense suspension of yeast phase cells on the chorioallantoic membrane. In addition, several hundred embryos were inoculated intravenously with both of these organisms. With H. capsulatum, widespread lesions of the viscera of the embryo were established. The infection was lethal to the embryo. Systemic lesions did not result in the embryo when cells of S. schenckii were injected intravenously. However, with both organisms plaques regularly developed when the inoculation was made onto the chorioallantoic membrane.

Four dozen 20 Gm. white male mice were each inoculated with 0.5 cc. of a dense suspension of the yeast phase cells of H. capsulatum and S. schenckii. Groups of these animals were killed at weekly intervals and biopsy specimens taken from appropriate tissues. The human material was collected from the dermatopathologic service of the University Hospital and came from two cases of histoplasmosis and three cases of sporotrichosis. All the tissues were stained with hematoxylin and eosin in the usual fashion. In addition the periodic acid-Schiff procedure (Hotchkiss-McManus stain) was employed. The staining procedure has been described elsewhere.[5] The latter stain is more or less specific for polysaccharides. Since the capsules of other micro-organisms are often rich in polysaccharides, the Hotchkiss-McManus stain seemed advantageous for this study. The best results with H. capsulatum were obtained when the periodic acid hydrolysis was carried out at 50 C. instead of at room temperature. Incidentally, it was often desirable in the present study to restain the original hematoxylin-eosin slides with the Hotchkiss-McManus stain. This was accomplished simply by immersing the hematoxylin-eosin slide directly into periodic acid. The hydrolysis brought about by the periodic acid simultaneously destained the hematoxylin-eosin preparation.

The in vivo forms of these organisms were prepared for electron microscopy as follows. Infected tissues were first ground with water in a mortar. The crude suspension was then differentially centrifuged with successive removal of the separates. These in turn were examined for their fungus content, and the separate containing the greatest number of organisms was used for study under the electron microscope. A drop of the appropriate separate was placed on the electron microscope screen and allowed to dry.

In performing the *Quellung* reactions, the suspensions of fungous cells from tissue were prepared as for electron microscopy. A drop of suspension was placed on the slide and allowed to dry. After this, a drop of homologous antiserum was added along with a drop of dilute methylene blue. The serum was used both undiluted and diluted 10 and 20 times. A cover slip

3. Collins, W. T.: Disseminated Ulcerating Sporotrichosis with Widespread Visceral Involvement: Report of a Case, Arch. Dermat. & Syph. **56**:523 (Oct.) 1947.

4. Moore, M.: Am. J. Path. **17**:103, 1941.

5. Kligman, A. M., and Mescon, H.: J. Bact. **60**:415, 1950.

was put on and immediately ringed with petrolatum. The H. capsulatum antiserum was obtained from infected guinea pigs and had a complement fixation titer of 1:80. The S. schenckii serum was obtained from immunized guinea pigs and had an agglutination titer of 1:320.

RESULTS

Histoplasma Capsulatum.—H. capsulatum had an identical appearance in chick, mouse and human tissue. The halos surrounding the basophilic central or eccentric mass in hematoxylin-eosin preparations were quite evident (fig. 1 *A, B* and *C*). The restaining of the same preparation with the Hotchkiss-McManus technique resulted in a strikingly different appearance. There was no evidence of a capsule. Under these conditions the organisms were seen to have a definite red-stained cell wall which was not evident in the hematoxylin-eosin preparations (fig. 1 *D* and *E*). The cytoplasm was diffusely stained pink with no internal differentiation. These findings were made with 10 minutes of periodic acid hydrolysis. With lesser duration of the periodic acid hydrolysis at room temperature the organism stained in a different fashion and provided a clue as to the real nature of the "capsule" seen in the slides stained with hematoxylin and eosin. With three minutes of hydrolysis at room temperature the organism had an appearance not unlike that seen when it was stained with hematoxylin; i. e., there was a definite central red-staining mass which appeared to be surrounded by a clear zone. With close scrutiny, however, one could definitely make out a cell wall peripheral to the clear zone (fig. 1 *F*.). The "capsule" or halo observed around the central mass with the Hotchkiss-McManus technique was thus seen to be contained within the cell wall and was not external to it. The so-called "capsule" of H. capsulatum in a hematoxylin-stained preparation therefore appeared to be an intracellular artefact probably produced by plasmolysis which caused the cytoplasm to shrink away from the cell wall. The artefact evidently arose because the cell wall was not well visualized with the usual stains, and the clear space did indeed appear to be outside the basophilic mass which was previously considered to be the cell itself. Therefore, only a part of the cell was seen with the routine stains. Because of this, the organisms appeared larger in the periodic acid-Schiff preparations. When the period of hydrolysis was gradually increased to 10 minutes, and the temperature was likewise increased to 50 C., the central staining mass seen with three minutes of hydrolysis became less conspicuous and finally disappeared. The cytoplasm stained a light pink.

The disappearance of the central staining mass with prolongation of the period of hydrolysis is probably accounted for by the production of soluble hydrolytic products which diffused away during the staining procedures. With 10 minutes of hydrolysis at 50 C., the organism was seen as a red oval ring and looked so unlike hematoxylin-eosin preparations that an experienced dermatopathologist was unable to recognize the organism after examining the slide. His inability to do so was also partly attributed to the unfamiliarly large size of the cells. It should also be pointed out that in Hotchkiss-McManus-stained preparations the intracellular position of the organisms is not evident, since the host cells do not stain.

The organisms were clearly visualized under the electron microscope. There was no evidence of a capsule (fig. 1 *G* and *H*). The central portion of the cell was denser than the periphery, an appearance probably arising from desiccation. Electron photomicrographs of the yeast phase obtained from a blood agar culture had a similar appearance.

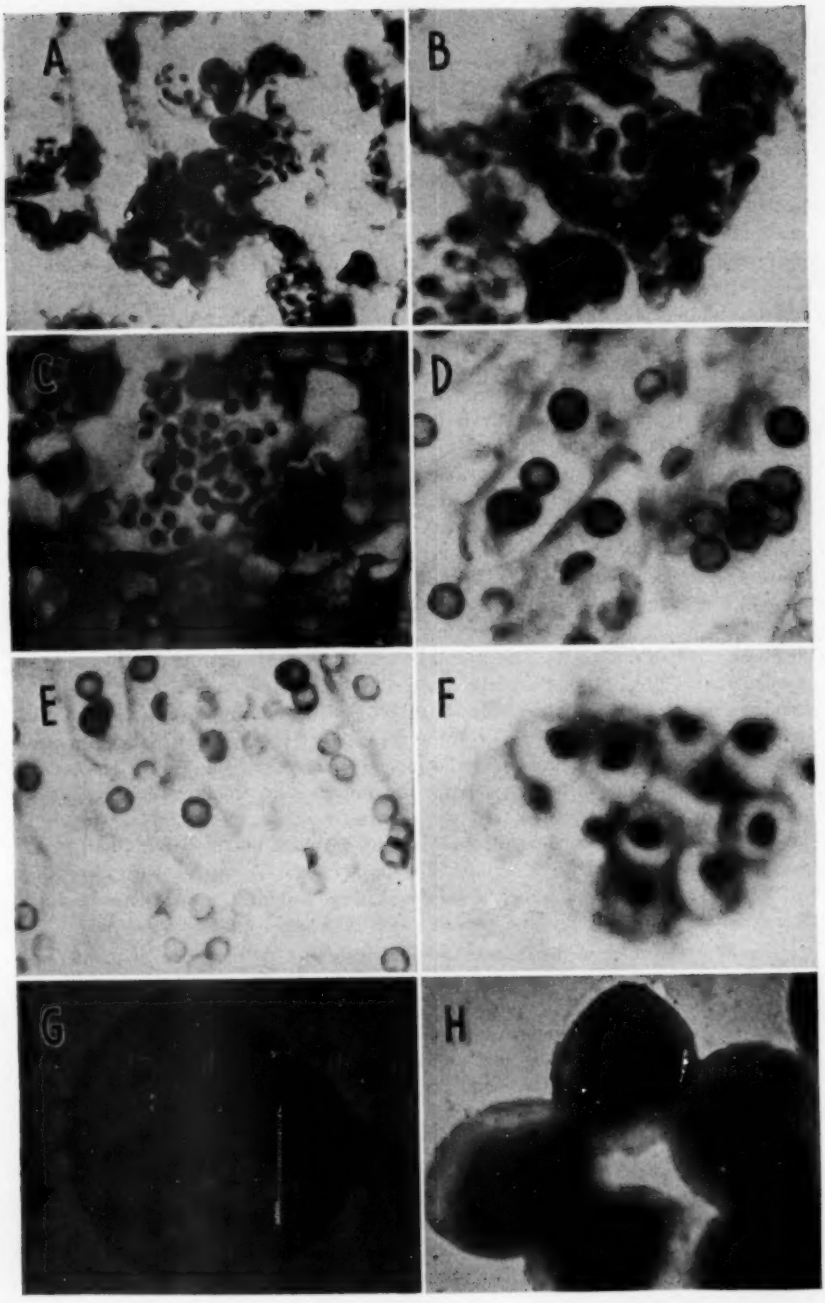

Figure 1

(See legends on opposite page)

Finally, there was no evidence of a quellung reaction when H. capsulatum cells obtained from tissue were mixed with homologous antiserum.

Sporotrichum Schenckii.—This organism seen in tissue is usually described as cigar-shaped.[6] This description is inaccurate. A review of preparations of experimental and human material showing sporotrichosis indicated quite clearly that the striking feature of S. schenckii cells in tissue was their polymorphism. They were variously oval, globose, elliptical, fusiform, bacilliform, pyriform, club-shaped, etc. The cells exhibited variation not only in size but in shape (fig. 2 *G*). Occasionally, large round cells, 15 microns in diameter, were observed. Such relatively huge cells were not infrequently observed in one of our human specimens. If this cell did not occur in association with other more typical cells, its identity would certainly not have been apparent. In both animal and human tissue many of the cells were within macrophages. Often the macrophages had all their extranuclear content filled with organisms (fig. 2 *D*). In tissues from two of our three cases of human sporotrichosis, however, only a few organisms were found. The rarity of organisms in human biopsy material is usual. In mice the parasites were exceedingly common. In both human and animal tissue, budding was observed usually in the form of elongate, fusiform herniations arising from one end of the cell. Sometimes, however, ovoid budding cells similar to those seen in the genus Candida were observed.

In the chorioallantoic membrane of the chick embryo, the polymorphism of the organism was even more marked. In addition to the forms described above, there were definite mycelial fragments, some of which were branched (fig. 2 *B*).

The chick embryo characteristically responds to fungous infections by the formation of a large number of irregular giant cells. Most of the organisms were con-

6. Dubos, R. J.: Bacterial and Mycotic Infections of Man, Philadelphia, J. B. Lippincott Company, 1948.

EXPLANATION OF FIG. 1

Fig. 1.—Histoplasma capsulatum: *A*, macrophages of the chorioallantoic membrane of the chick embryo containing H. capsulatum; hematoxylin-eosin; × 700. The parasites are surrounded by clear halos (the so-called "capsules").

B, high power view of the field shown in *A*; hematoxylin eosin; × 1,550. The "capsules" seem quite definite.

C, cells of H. capsulatum in human tissue; hematoxylin-eosin; × 950. The halos are clearly evident, but close inspection shows a distinct outer ring bordering the halo. This ring is the cell wall, which ordinarily is not this well visualized in hematoxylin-eosin preparations.

D, cells of H. capsulatum in human tissue; periodic acid-Schiff stain; × 1,550. In this type of preparation the cell wall is prominently defined. No capsule is evident. There is no internal differentiation. Note that although the magnification is the same as in *B*, the organism appears larger. This is because the cell wall is not seen in *B*.

E, cells of H. capsulatum in human tissue; periodic acid-Schiff stain; × 1,300. Many of the organisms stain lightly. Nonetheless, the cell wall is still well defined, and there is no suggestion of a capsule. The intracellular position of the parasites is not evident, since the host cells are not stained.

F, cells of H. capsulatum in human tissue; periodic acid-Schiff stain; × 2,850. This specimen was hydrolyzed for only three minutes in periodic acid. The result is not unlike that of a well stained hematoxylin-eosin preparation. Halos are again evident. These suggest capsules except that the cell walls surrounding the clear halos can be visualized. The dark, centrally placed mass is probably retracted cytoplasm which has shrunk away from the cell wall leaving a clear space.

G, cell of H. capsulatum from mouse tissue; electron photomicrograph; × 11,000. This cell appears to be initiating a bud. There is no capsule.

H, cells of H. capsulatum from a blood culture at 37 C.; electron photomicrograph; × 7,500. These cells are not encapsulated.

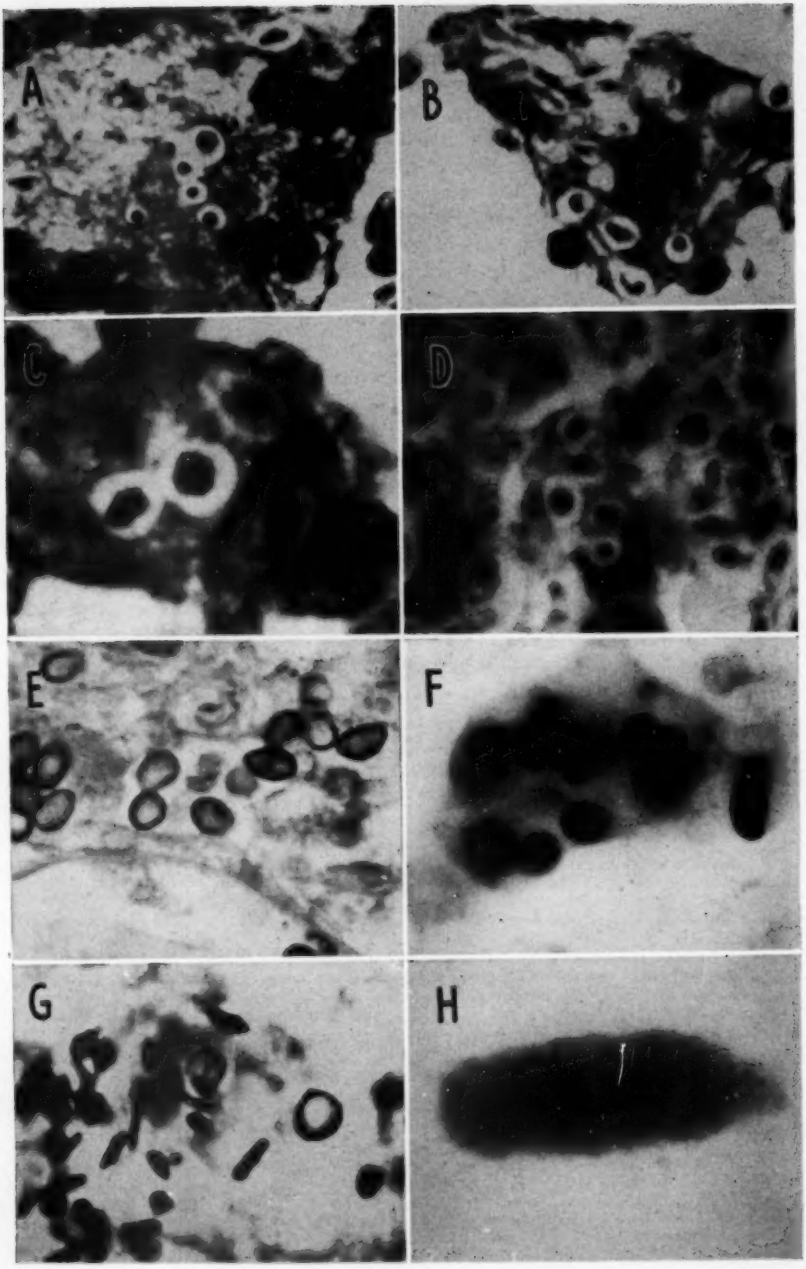

Figure 2

(See legends on opposite page)

tained in these large phagocytic cells (fig. 2 *F*). In the hematoxylin and eosin-stained preparations the parasites appeared to have particularly prominent "capsules" in this particular tissue. A very definite clear zone, which was often quite large, could be seen surrounding the cells (fig. 2 *A*, *B*, and *C*). They appeared to possess a definite capsule. With the Hotchkiss-McManus stain, however, the presence of a capsule could not be confirmed (fig. 2 *E* and *F*). Indeed the "capsule" appeared to be an artefact arising in much the same way as with H. capsulatum. The definitive staining of the cell wall in the Hotchkiss-McManus specimens made it clear that the so-called "capsule" was included within the cell and was not an extracellular component.

In the mouse, numerous extracellular organisms could be observed in the central portion of the abscesses. In addition, many organisms occurred intracellularly in the epithelioid cells which characteristically formed a broad zone around the abscess. The intracellular organisms in this situation also appeared encapsulated after hematoxylin-eosin staining, although this appearance was less clearcut than in the chick embryo (fig. 2 *D*). When the same preparations were studied by the Hotchkiss-McManus technique, the factitial nature of the capsules became apparent. The cytoplasm stained homogenously red with no internal differentiation and was surrounded by a darker staining red wall (fig. 2 *E*).

The quellung reaction could not be observed in cells exposed to undiluted homologous antiserum or in antiserum diluted 1 : 10 or 1 : 20. Attempts were made to demonstrate the capsule by suspending organisms obtained from tissues in india ink under a cover slip, a procedure which is most useful in demonstrating the capsule of Cryptococcus neoformans. This, too, failed.[7]

There was no evidence of a capsule in the organisms viewed under the electron microscope (fig. 2 *H*).

7. Since submitting the manuscript for publication, we have become aware of the work of Norden, who also was unable to demonstrate a quellung reaction with S. schenckii. He is apparently convinced, however, that this organism is encapsulated (Norden, A.: Sporotrichosis: Clinical and Laboratory Features and a Serologic Study in Experimental Animals and Humans, Acta path. et microbiol. scandinav., supp. 89, Copenhagen, Ejnar Munksgaard, 1951).

EXPLANATION OF FIG. 2

Fig. 2.—Sporotrichum schenckii: *A*, giant cell of the chorioallantoic membrane of the chick embryo containing S. schenckii; hematoxylin-eosin; × 750. The intracellular parasites appear to be distinctly encapsulated.

B, cells of S. schenckii in a macrophage of the chorioallantoic membrane; hematoxylin-eosin; × 750. Some hyphal elements are present. These, too, appear to be encapsulated.

C, two cells showing what seem to be unmistakable "capsules"; hematoxylin-eosin; × 1,200.

D, cells of S. schenckii in epithelioid cells of mouse tissue; hematoxylin-eosin; × 900. The host cells are crowded with the intracellular parasites which, because of their "capsules," strikingly resemble H. capsulatum.

E, cells of S. schenckii in mouse tissue; periodic acid-Schiff stain; × 900. In contrast to *D* in which the cell walls were not stained, the cell walls are now brilliantly defined, and the so-called "capsules" have disappeared. These organisms also look larger than those in *D*, although they were taken at the same magnification.

F, macrophage of chorioallantoic membrane, containing S. schenckii; periodic acid-Schiff stain; × 1,050. The "capsules" are no longer evident, although, as shown in *A*, *B* and *C*, halos are especially prominent in chick membrane tissue stained with hematoxylin and eosin.

G, cells of S. schenckii in human tissue; periodic acid-Schiff stain; × 800. There is great variation in the size and the shape of the organisms. They are not encapsulated, however.

H, cells of S. schenckii from mouse; electron photomicrograph; × 3,500. There is no capsule.

COMMENT

In this study no evidence could be found for the existence of a capsule in the tissue phases of H. capsulatum or S. schenckii. The seeming presence of a capsule is due apparently to retraction of the cytoplasm from the cell wall and the failure of the cell wall to be well stained by the usual histologic procedures. After the factitial nature of the "capsule" of H. capsulatum was disclosed, the preparations stained with hematoxylin and eosin were minutely examined. One could, on occasion, make out a faintly staining cell wall, so that even with this stain the capsule could be shown to be an artefact (fig. 1 *C*).

The findings in this study with regard to the capsule of S. schenckii are not in accord with those of Neill and his associates.[2] These workers believed they were able to demonstrate a capsule by means of the quellung reaction. However, these investigators stated that "when Sporotrichum cells prepared from mice were mixed with Sporotrichum anti-serum, capsules with a distinctly outlined and relatively dark *outer border* were clearly evident; whereas when mixed with a control antiserum or with salt solution, no definite capsules were apparent." Subsequently, they stated that "Sporotrichum cells which showed capsules in ordinary wet mounts without serum were frequent in some materials." In other words, it would appear that these investigators were on occasion able to observe capsules even in the absence of homologous antiserum. If their published figures are studied, one does indeed see that the object labeled "capsule" is "distinctly outlined with a relatively dark outer border." This border in fact seems to be a rather distinct structure with definite breadth and is possibly the cell wall. If this is the case, the clear space which it surrounds can no longer be maintained to be a capsule. In their published photographs, cells with well developed "capsules" appear to be of the same size as nonencapsulated cells, a feature which again suggests that the border around the "capsule" is the cell wall. It should be pointed out that the organisms studied by Neill and his group were found in smears obtained by rubbing the peritoneal surfaces of infected mice. The organisms were not studied in tissue sections.

In our numerous attempts to demonstrate a quellung reaction with S. schenckii, occasional cells were seen which resembled the "encapsulated" organisms described by Neill and his associates. In these instances, however, the outer border was interpreted to be the cell wall and not the periphery of the capsule. Occasionally, one sees written descriptions of the tissue phase of Blastomyces dermatitidis in which the organism is said to have a thick capsule. Plasmolysis with the cytoplasm retracting from the cell wall is a prominent feature of this organism. The mistaken notion that B. dermatitidis has a capsule derives from the same phenomenon described in this paper for S. schenckii and H. capsulatum.

SUMMARY

Chick embryo, mouse tissue and human tissue infected with Histoplasma capsulatum and Sporotrichum schenckii were studied histologically with hematoxylin-eosin and Hotchkiss-McManus stains. The "capsules" seen in sections stained with hematoxylin and eosin could not be demonstrated with the more definitive Hotchkiss-McManus technique. The study indicated that the so-called capsules were artefacts brought about by plasmolysis of the cytoplasm due to fixation.

The quellung reaction could not be demonstrated with either H. capsulatum or S. schenckii.

Electron microscope studies of these organisms failed to reveal a capsule.

HYPERTENSIVE CARDIOVASCULAR DISEASE

Vascular Lesions of Dogs Maintained for Extended Periods Following Bilateral Nephrectomy or Ureteral Ligation

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L. B. TURNER, M.D.

AND

ARTHUR GROLLMAN, M.D., Ph.D.

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IN PREVIOUS papers we have reported [1] the development of hypertension in dogs following bilateral nephrectomy. These animals, surviving an average of less than 10 days, revealed at autopsy necrosis and hemorrhages of the heart and necrosis of the media of small arteries and arterioles.[1b] A tendency toward hyalinization of the media of small arteries and arterioles, however, was noted in animals surviving more than 10 days following nephrectomy. Subsequently, it was shown that these necrotic lesions also occurred following certain manipulations of the kidneys not associated with an elevation of blood pressure.[2] As a method of prolonging the life of the nephrectomized dog, peritoneal lavage has made it possible to maintain such animals alive for as long as 70 days.[3] The vascular and cardiac lesions encountered under these conditions differ from those previously described and resemble those generally considered as the classic vascular lesions of the hypertensive state of humans. These lesions, as well as those observed after bilateral ligation of the ureters, are the subject of the present report.

METHODS

Mongrel dogs of average size were nephrectomized or their ureters were ligated as described elsewhere.[1a] They were then maintained on a salt-free diet and subjected to peritoneal lavage by the procedure described elsewhere.[3] Blood pressures were determined by direct puncture of the femoral artery.[4] The animals were killed at varying periods or the tissues were removed

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Dr. Turner is Fellow of the Dazian Foundation for Medical Research.

This work was supported by a grant from the Life Insurance Medical Research Fund.

From the Departments of Experimental Medicine and Pathology, Southwestern Medical School of the University of Texas.

1. (*a*) Grollman, A.; Muirhead, E. E., and Vanatta, J.: Am. J. Physiol. **157**:21, 1949. (*b*) Muirhead, E. E.; Vanatta, J., and Grollman, A.: Hypertensive Cardiovascular Disease: Experimental Study of Tissue Changes in Bilaterally Nephrectomized Dogs, Arch. Path. **48**:234 (Sept.) 1949.

2. Muirhead, E. E.; Grollman, A., and Vanatta, J.: Hypertensive Cardiovascular Disease ("Malignant Hypertension"): Changes in Canine Tissues Induced by Various Manipulations of the Kidney, with Special Reference to Vascular and Myocardial Lesions, Arch. Path. **50**:137 (Aug.) 1950.

3. Grollman, A.; Turner, L. B., and McLean, W.: Intermittent Peritoneal Lavage in Nephrectomized Dogs and Its Application to the Human Being, A. M. A. Arch. Int. Med. **87**:379 (March) 1951.

4. Grollman, A.: Am. J. Physiol. **147**:647, 1946.

as soon as possible after death, fixed in formaldehyde solution U. S. P., and stained with hematoxylin and eosin.

A total of 32 dogs comprise the present series. Of these, 22 were bilaterally nephrectomized and 10 were subjected to bilateral ureteral ligation.

RESULTS

Table 1 summarizes the type of cardiovascular lesions observed in the present study. Hyperacute and acute arterial lesions are grouped together. The term "hyperacute" is used to designate the necrotic lesions of the media previously described,[1b] which are characterized mainly by necrosis and eosinophilic smudging or smearing of the media. In such lesions the necrotic media usually spreads toward the adventitia, although at times the material spills into the lumen, giving

TABLE 1.—*Classification of the Cardiovascular Lesions Observed in Nephrectomized Dogs Surviving for Periods of One to Ten Weeks*

I.	**Heart**
	A. Necrosis and hemorrhage (acute) involving:
	1. Isolated fibers
	2. Groups of fibers—with or without neutrophilic leukocytes (frequently subendocardial)
	B. Reparative process (subacute and chronic) with:
	1. Granulation tissue and inflammatory cells (lymphocytes, macrophages and occasionally Anitschkow's cells)
	2. Hyaline scars
	3. Calcification of necrotic fibers (variable)
II.	**Small Arteries and Arterioles**
	A. Media
	1. Necrosis and smudging (hyperacute)
	2. Necrosis and early hyalinization—pyknosis and karyorrhexis variable (acute)
	3. Hyalinization—circumferential or segmental, concentric or eccentric (subacute and chronic)
	4. "Hypertrophy" and "hyperplasia" (chronic)
	B. Intima
	1. Thickening of internal elastic lamina
	2. Subendothelial hyalinization
	3. Subendothelial fibrosis (endarteritic type)
	4. Endothelial proliferation
	C. Combination of atrophy of media and thickening of intima
III.	**Aorta: Partial degeneration of media (rarely observed)**

rise to various grades of thrombonecrosis.[5] The term "acute arterial lesion" is used to designate the early hyaline change of the media with or without the presence of nuclear remnants such as pyknosis or karyorrhexis (figs. 1 *A* and 3 *A* and *B*). By "early hyaline" is meant that the necrotic media appears to be more solid in consistency. Instead of disintegrating as in the hyperacute lesion, the media retains its shape and its substance changes into closely adherent blocks or masses of hyaline appearance. This lesion, encountered only occasionally previously,[2] became frequently evident in animals surviving beyond 10 days. For its genesis two possible explanations immediately present themselves. It may represent a subsequent alteration of the hyperacute, smudged type of necrosis; or it represents, more likely, a slower development of the necrosis. The latter interpretation is supported by the apparent slower degradation of the nuclear substance of the media as revealed by the lingering pyknosis and karyorrhexis.

The subacute and chronic arterial and arteriolar lesions were represented by different morphologic patterns as follows:

5. Muirhead, E. E.; Grollman, A., and Turner, L. B.: To be published.

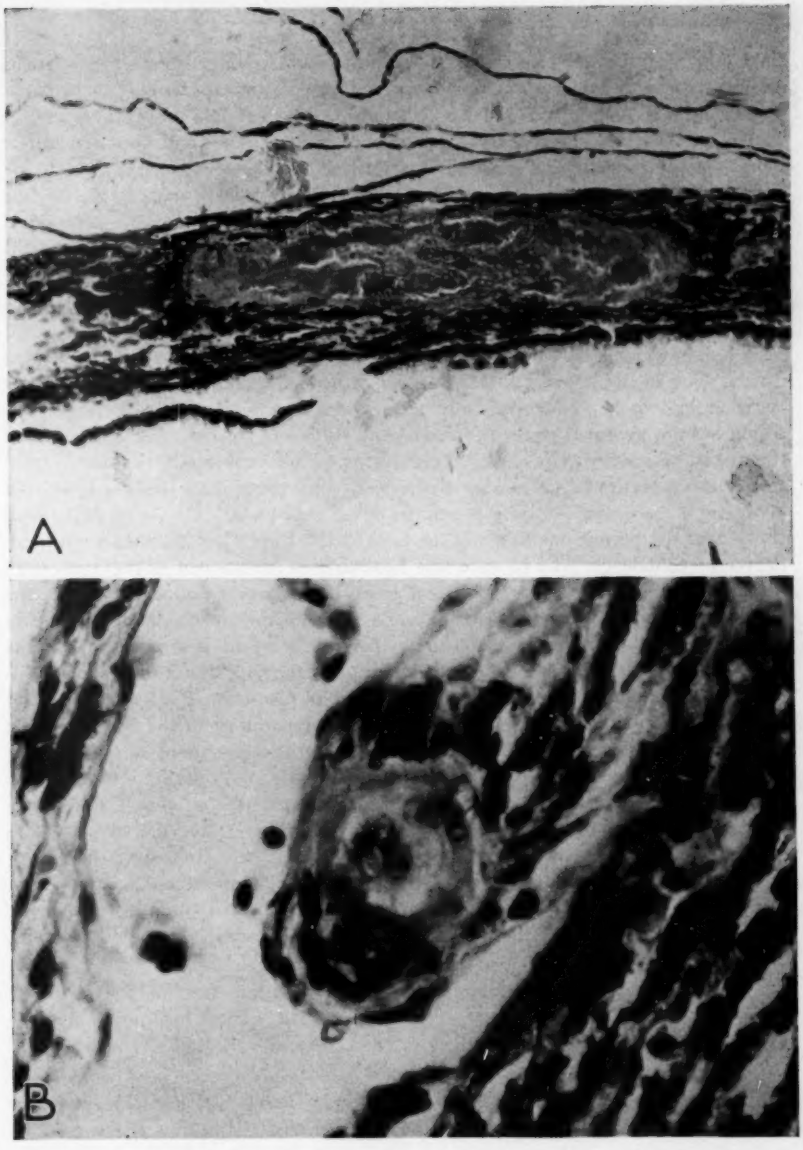

Fig. 1.—Lesions of the arterioles and smaller arteries as observed in bilaterally nephrectomized dogs:

A (dog 19, killed 38 days after nephrectomy; blood pressure, 200 mm. Hg), acute and subacute lesion of a small artery of the eye (iris), showing hyalinization of the vessel wall involving mainly the media. Fragments of nuclei persist in the media, and endothelial proliferation of an irregular nature is evident. × 130.

B (same dog as in *A*), subacute lesion of an arteriole in the choroid coat of the eye, showing partial hyalinization of the wall involving mainly the media and an apparent increase in endothelial cells. × 765.

Total hyalinization of the vessel wall, the most commonly observed change, frequently involved the entire circumference of a vessel and was more often eccentric than concentric (figs. 1 *A* and *B*, 2 *A* and *B*, 3 *B*, 4 *A* and 5 *A* and *B*). The lumens of these vessels were usually narrowed in appearance and displaced to one side. At this apparent end stage of the arteriolar sclerosis, one could not determine from isolated observations which layer of the wall contributed most to the hyaline structure. It was certain, however, that the smooth muscle of the media had either completely or almost completely disappeared as a definable structure by the time complete hyalinization had occurred.

Another type of subacute or chronic lesion consisted of a thickening of the arteriolar wall coincident with increased cellularity of the wall (figs. 4 *B* and 6 *A*). This lesion, which has been termed "hyperplasia" of arterioles, was associated with a narrowing of the lumen. Subendothelial or medial hyalinization was present on occasion in vessels so involved. In the present observations this "hyperplastic" lesion has not appeared to result from a proliferation of smooth muscle elements of the media. As shown elsewhere,[5] the elements of this proliferative lesion have the tinctorial properties of connective tissue and, consequently, the lesions appear to represent a concentric fibrous replacement of the vessel wall. However, the present study does not permit one to apply the term "hypertrophy" of the media with any degree of confidence. To be sure, the hyalinization, mentioned above, represents an increase in size of the media in focal areas, but it does not appear to justify the application of the term "hypertrophy." As previously described,[1b] an increase in size of single or groups of smooth muscle fibers of the media is a common observation. The increase in bulk of the sarcoplasm takes the appearance of hyaline swelling with or without nuclear alteration in the form of pyknosis. The hyaline swelling could be readily interpreted either as a change preparatory to the hyperacute or necrotic lesion, that is, as a prenecrotic change, or as a precursor of the acute or early hyaline lesion. At any rate, the hyaline swelling could not be interpreted as indicative of an increase in the functional elements of the smooth muscle sarcoplasm and hence did not warrant the designation "hypertrophy." Yet, the term "hypertrophy" of the media cannot be entirely dismissed from this discussion, since there appeared to exist a prominence of the terminal arterioles or precapillary vessels in many tissues (fig. 4 *A*). This prominence seemed to result from an increase in the size of the few smooth muscle fibers in the wall of these vessels in the absence of a hyaline change.

The accumulation of a hyaline substance, just beneath the endothelial lining, appeared to be of two separate types: (1) a partial or focal hyalinization of the media extending toward the endothelium (fig. 1 *B* and 4 *A*) and (2) a deposition of hyaline substance separate from the media (fig. 6 *A*).

The subendothelial accumulation of connective tissue had features similar to the subendothelial hyalinization. It either merged with the fibrous replacement of the media or occurred alone (fig. 6 *A*). The accumulation of either subendothelial hyaline substance or subendothelial connective tissue or both was usually associated with atrophy and disappearance of the media as an organized component of the vessel wall.

Endothelial proliferation occurred usually in conjunction with complete or partial hyalinization of the wall, usually of the media (figs. 1 *A* and *B*). This rare

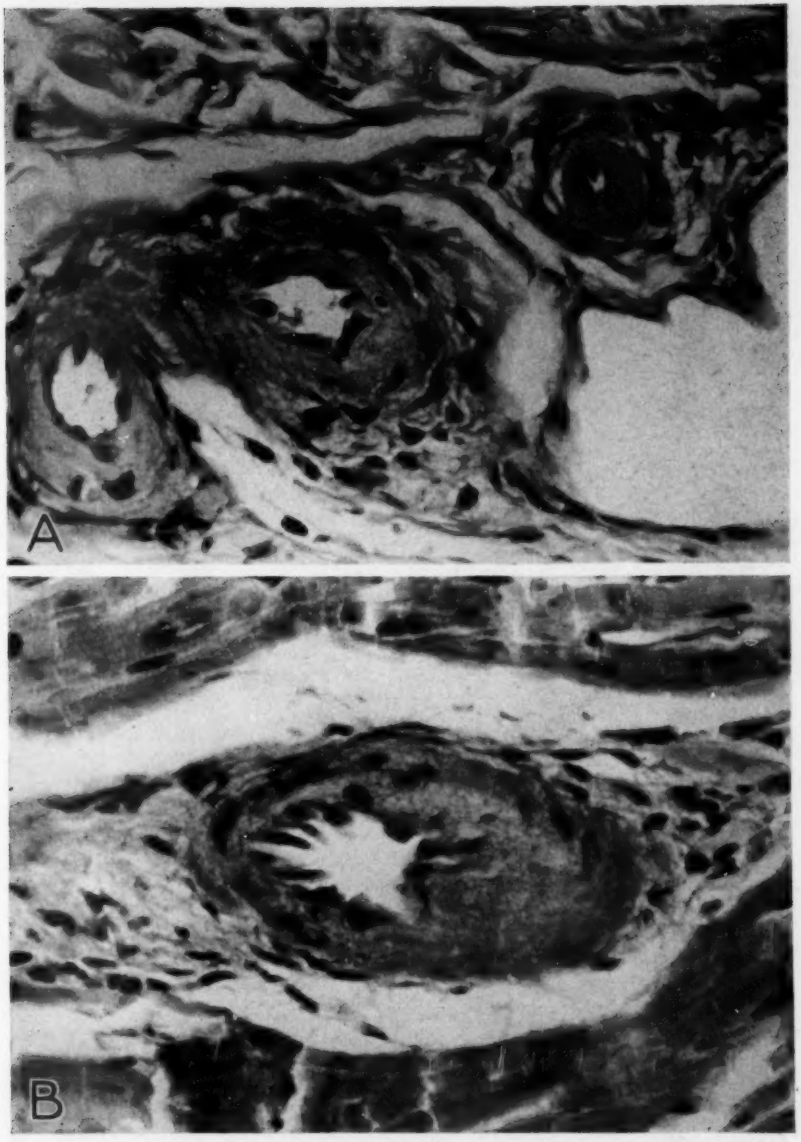

Fig. 2.—Lesions of the arterioles and smaller arteries as observed in bilaterally nephrectomized dogs:

A (dog 16, 40 days after nephrectomy; blood pressure, 175 mm. Hg), chronic lesions of the arterioles in the submucosa of the colon, showing total hyalinization and thickening of the wall and narrowing and displacement of the lumen. × 556.5.

B (same dog as in *A*), chronic lesion of an arteriole in the myocardium, showing nearly total hyalinization of the wall; a few smooth muscle fibers remain in the media. × 608.5.

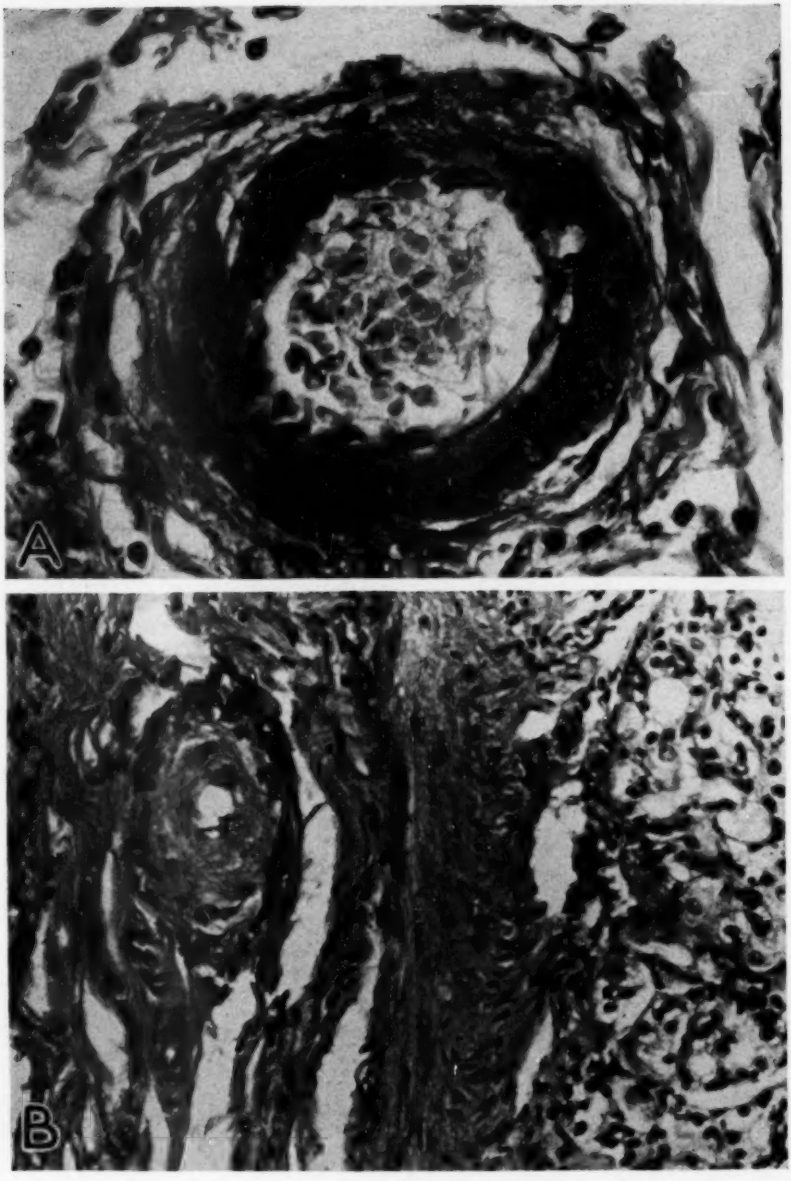

Fig. 3.—Arteriolar lesions observed in bilaterally nephrectomized dogs:

A (same dog as in fig 1 *A*), acute lesion of an arteriole in the submucosa of the small intestine, showing early hyalinization of the media, pyknosis and karyorrhexis. The endothelium is intact; a few smooth muscle fibers of the media have intact nuclei. × 666.5.

B (dog 14, killed 70 days after nephrectomy; blood pressure, 165 mm. Hg), acute and subacute lesions of an arteriole of the submucosa of the stomach, showing hyalinization and thickening of the wall and a few nuclear remnants. × 409.5.

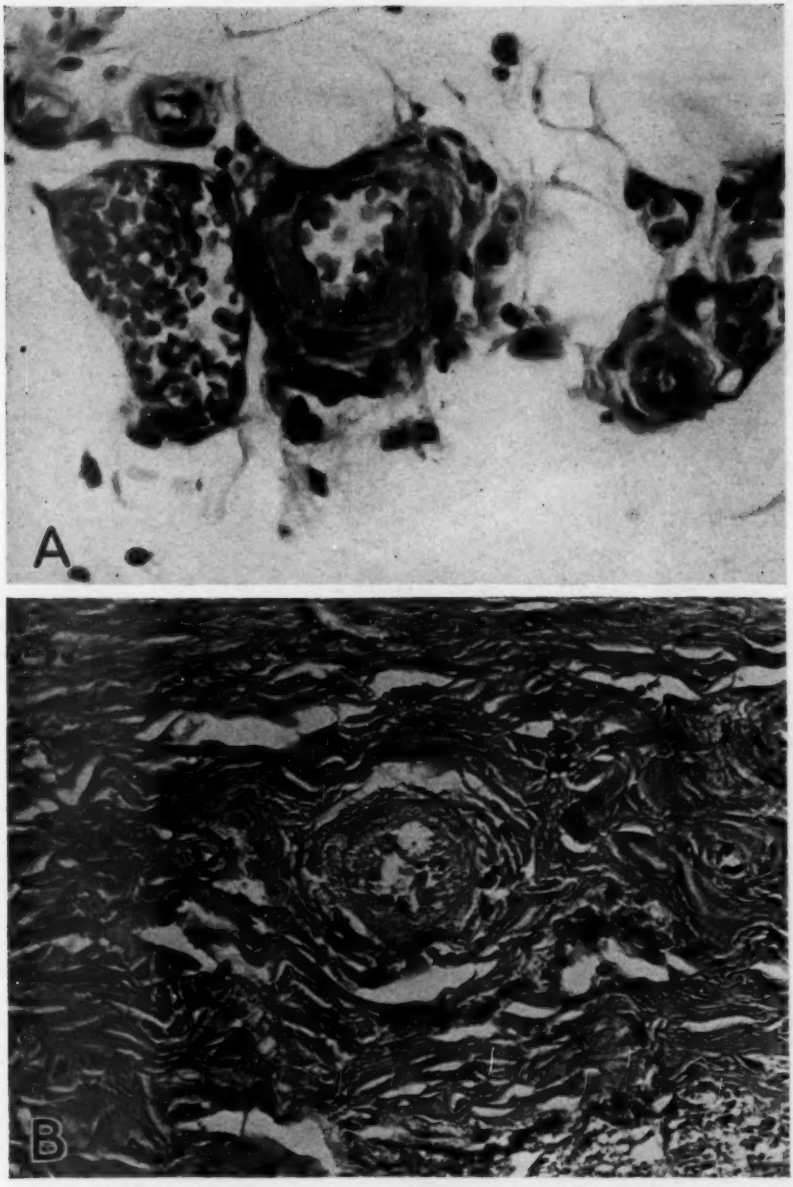

Fig. 4.—Arteriolar lesions observed in bilaterally nephrectomized dogs:

A (same dog as in fig. 3*B*), chronic lesion of an arteriole of the mesentery, showing total hyalinization of a major segment of its circumference. There are also three precapillary vessels showing prominence or "hypertrophy" of their smooth muscle. × 666.5.

B (dog 29, 31 days after nephrectomy; blood pressure, 180 mm. Hg), chronic lesion of an arteriole of the submucosa of the small bowel, showing a thickened wall containing vacuoles and having a partly hyaline and partly granular appearance. Note the subendothelial connective tissue cells. × 333.

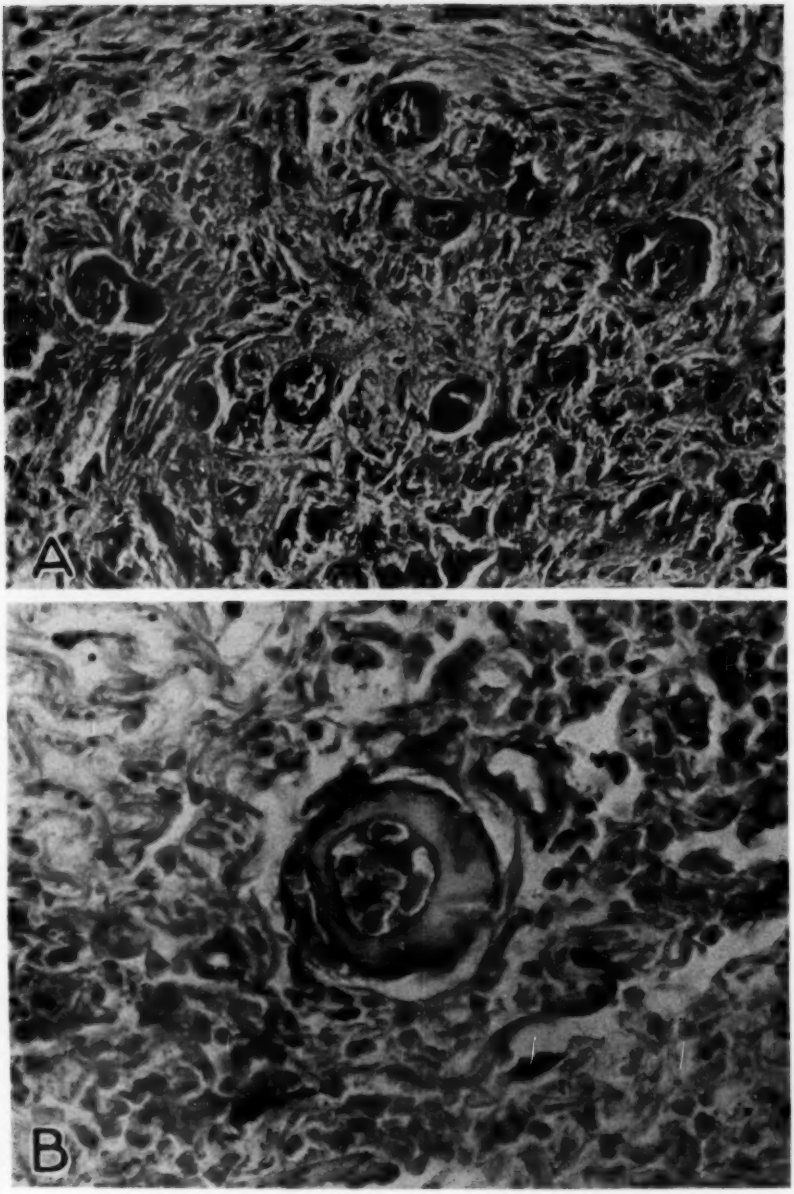

Fig. 5.—Arteriolar lesions observed in bilaterally nephrectomized dogs:

A (dog 23, 34 days after nephrectomy; blood pressure, 200 mm. Hg), chronic lesion of the arterioles of the medulla of the adrenal gland, showing total hyalinization with narrowed and eccentric lumens. × 143.

B (dog 10, 34 days after nephrectomy; blood pressure, 155 mm. Hg), chronic lesion of an arteriole of the submucosa of the stomach, showing total hyalinization and an eccentric lumen. × 838.

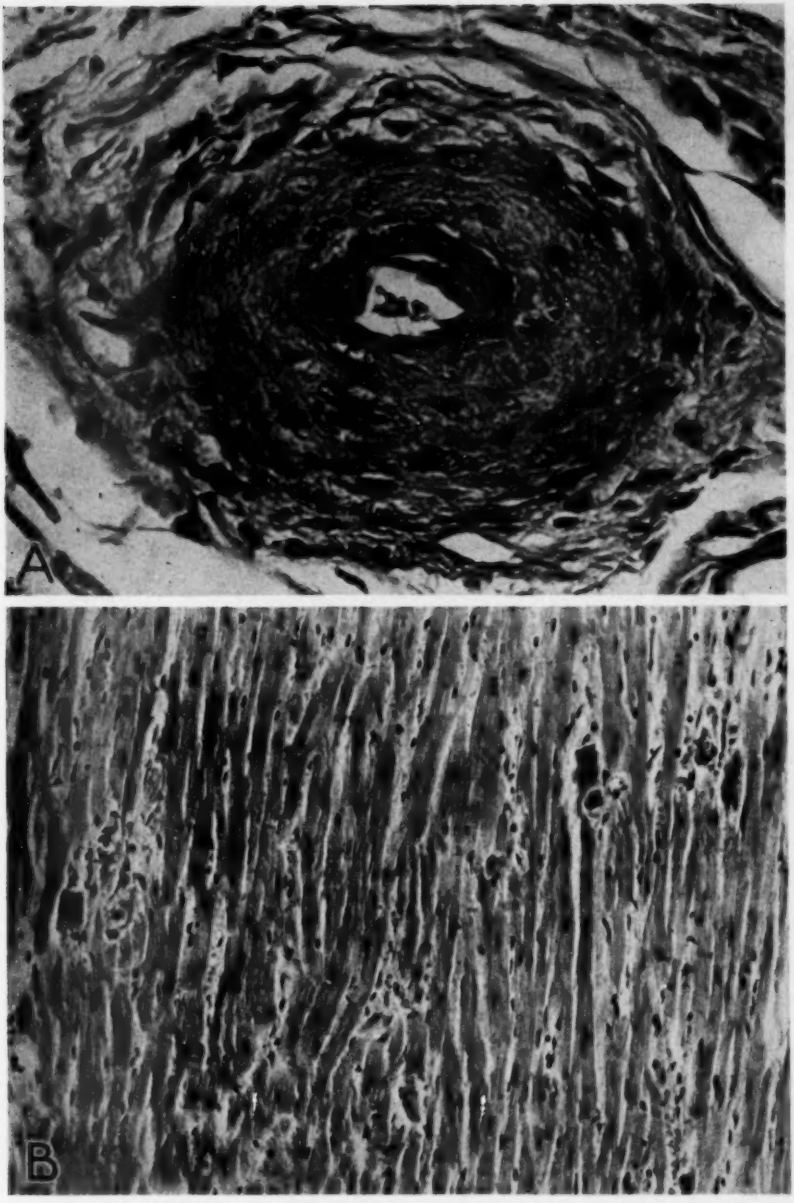

Fig. 6.—Lesions of an arteriole and of the myocardium as observed in nephrectomized dogs:

A (same dog as in fig. 2*A*), subacute and chronic lesions of an arteriole in the submucosa of small bowel, showing a thickened and hypercellular wall and a narrowed lumen. There is also a collection of subendothelial hyaline. The cellularity is interpreted as fibrous replacement of the vessel wall rather than as medial hyperplasia. × 582.

B (dog 42, 30 days after nephrectomy; blood pressure, 165 mm. Hg), necrosis of isolated fibers of the myocardium. Note the condensed appearance of the sarcoplasm and the pyknosis. × 227.

observation included an irregular piling-up of endothelial cells toward the lumen. We have not been able to distinguish a concentric proliferation of endothelium associated with narrowing of the lumen. It may be that the term "endothelial pro-

TABLE 2.—*Incidence of Lesions Observed in Twenty-Two Bilaterally Nephrectomized Dogs and in Ten Dogs Subjected to Bilateral Ureteral Ligation*

Organ	Pathological Changes	Incidence of Lesions: Nephrectomized	Incidence of Lesions: Ureteral Ligation
Heart			
	Necrosis	13	4
	Repair (granulation tissue and fibrosis)	9	0
	Necrosis of arterioles	4	3
	Hyalinization of arterioles	3	0
	Calcification	4	0
Aorta			
	Degeneration	2	1
Lungs			
	Hyperemia, edema, capillary hemorrhages	12	7
	Bronchopneumonia	2	2
Liver			
	Hemosiderosis	10	0
	Hyperemia and centrolobular damage	9	6
	Necrosis of arterioles	1	0
	Hyalinization of arterioles	1	0
Spleen			
	Hemosiderosis	17	8
Stomach			
	Necrosis of arterioles	4	2
	Hyalinization of arterioles	6	1
	Edema of submucosa	3	1
	Chronic peritonitis	2	2
Bowel			
	Necrosis of arterioles	5	2
	Hyalinization of arterioles	5	0
	Focal necrosis	1	1
	Hemorrhages	2	2
	Chronic peritonitis	10	5
Pancreas			
	Hyalinization of arterioles	1	0
	Focal necrosis	2	0
Adrenals			
	Necrosis of arterioles	1	0
	Hyalinization of arterioles	3	0
Kidneys			
	Papillary necrosis and tubular degeneration	0	8
Urinary bladder			
	Necrosis of arterioles	1	1
	Hyalinization of arterioles	2	0
	Hemorrhages	0	7
	Edema	1	1
Brain			
	Hyperemia, edema, hemorrhages	8	3
Lymph node			
	Hemosiderosis	8	4
	Erythrophagocytosis	4	4

liferation" used by others has included the disturbance we have designated as subendothelial fibrous accumulation.

Although most of these changes could be observed with hematoxylin and eosin stain, they were more precisely delineated by their tinctorial properties with special stains.[5]

As shown in table 2, the arteriolar involvement was noted in many different tissues throughout the body and was absent notably only in the pulmonary vessels. The most common site of involvement was the gastrointestinal tract but the adrenal gland, the urinary bladder and the eyes were definitely involved in several animals.

In the eye these findings were especially interesting, since changes in this location are so intimately associated with hypertension as observed clinically. As shown in figure 1 *A* and 1 *B*, the arterioles of the iris and choroid showed definite changes. The most common change was focal medial hyalinization, although at times total hyalinization of the vessel wall was noted. Endothelial proliferation was observed in certain of these arterioles in the fundus. Associated with these vascular lesions, focal hemorrhages and edema of the choroid coat and retina occurred, giving these structures a fibrinoid appearance.

In three dogs, degenerative changes of the media of the aorta, which appeared loose, frayed and edematous, were observed. The edematous zones had a bluish granular appearance (fig. 7 *A*). Although this change suggested Erdheim's degeneration superficially, this suggestion may be open to question.

The cardiac lesions were also of acute, subacute and chronic types. The acute lesions, identical to those previously described,[6] were characterized by necrosis and hemorrhage. The necrosis was mostly subendocardial and involved especially the left ventricle near the apex and the papillary muscles. The necrosis began as lumpy granular foci in the fibers, followed by disintegration and hemorrhage. On occasions the granular change involved isolated segments of a fiber (fig. 6 *B*), although usually groups of fibers were so affected. The subacute lesion, consisting of the early phase of repair, was represented by necrotic areas partially or completely replaced by granulation tissue (fig. 8 *A*). The granulation tissue contained inflammatory cells, usually macrophages and lymphocytes. Rarely clusters of Anitschkow's cells were observed near an arterial branch. This early repair was noted during the second week and became especially evident after 10 days. After three weeks, advanced scarring was evident. By 30 days these scars were of hyaline consistency, having few cells, scattered lymphocytes and macrophages, hemosiderin pigment and occasionally capillary remnants (fig. 8 *B*). Most of the scars were detected by the microscopic study, but tiny linear gray scars and areas of increased resistance to sectioning by the knife were noted grossly.

As reported previously,[6] certain changes, apparently due to terminal circulatory failure, were also observed (table 2). These included pulmonary hyperemia, edema and scattered capillary hemorrhages; hyperemia of the liver with centrolobular damage, usually in the form of central atrophy, and hyperemia, edema and capillary hemorrhages of the brain. In addition, on four occasions calcification of necrotic cardiac fibers was extensive, and once there was metastatic calcification of the pulmonary alveolar walls. Evidence of bronchopneumonia was not common. Hemosiderosis of spleen, lymph nodes and liver was outstanding. The spleen showed the greatest concentration of hemosiderin (fig. 9 *A*). In the liver the hemosiderin was mainly deposited in the von Kupffer cells. All three of these structures, but especially the lymph nodes, revealed erythrophagocytosis. Intact red blood cells were seen within the cytoplasm of reticuloendothelial cells, either free in sinusoids or attached to the lining or inner structure, frequently alongside granules of hemosiderin (fig. 9 *B*). The adrenal glands were intact in most animals and contained ample lipid within the cortex. The zona glomerulosa was hypertrophied in most instances (fig. 7 *B*). About half of the animals showed varying degrees of fibrosis and chronic inflammation of the serosa of the abdominal viscera. This chronic peritonitis, on occasions, caused partial intestinal obstruction.

6. Muirhead, Vanatta and Grollman.[1b] Muirhead, Grollman and Vanatta.[2]

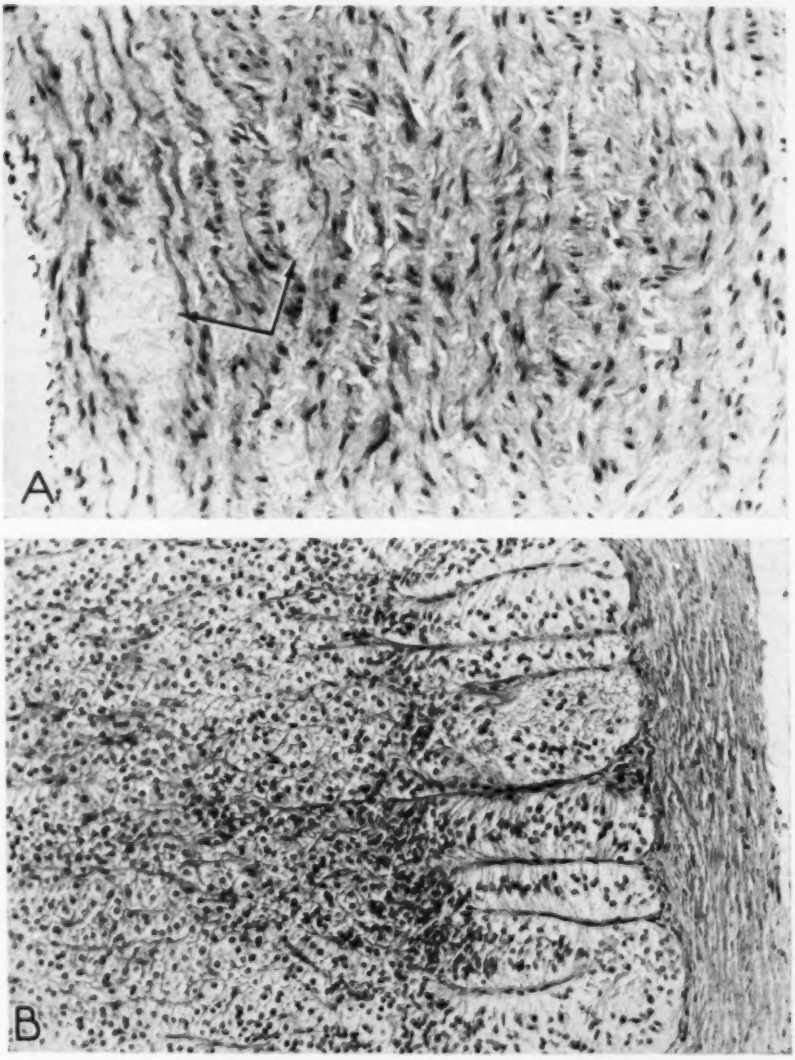

Fig. 7.—Microscopic appearance of aorta and adrenal cortex of bilaterally nephrectomized dogs:

A (same dog as in fig. 2 *A*), aorta showing foci of degeneration of the media. Note the foci near the intima, indicated by the arrows, showing the medial elements separated by a granular precipitate which in the hematoxylin and eosin stain is of blue color. × 200.

B (dog 1, 13 days after nephrectomy; blood pressure, 180 mm. Hg), adrenal cortex showing hypertrophy of the zona glomerulosa and the presence of an adequate amount of lipid throughout the cortex.

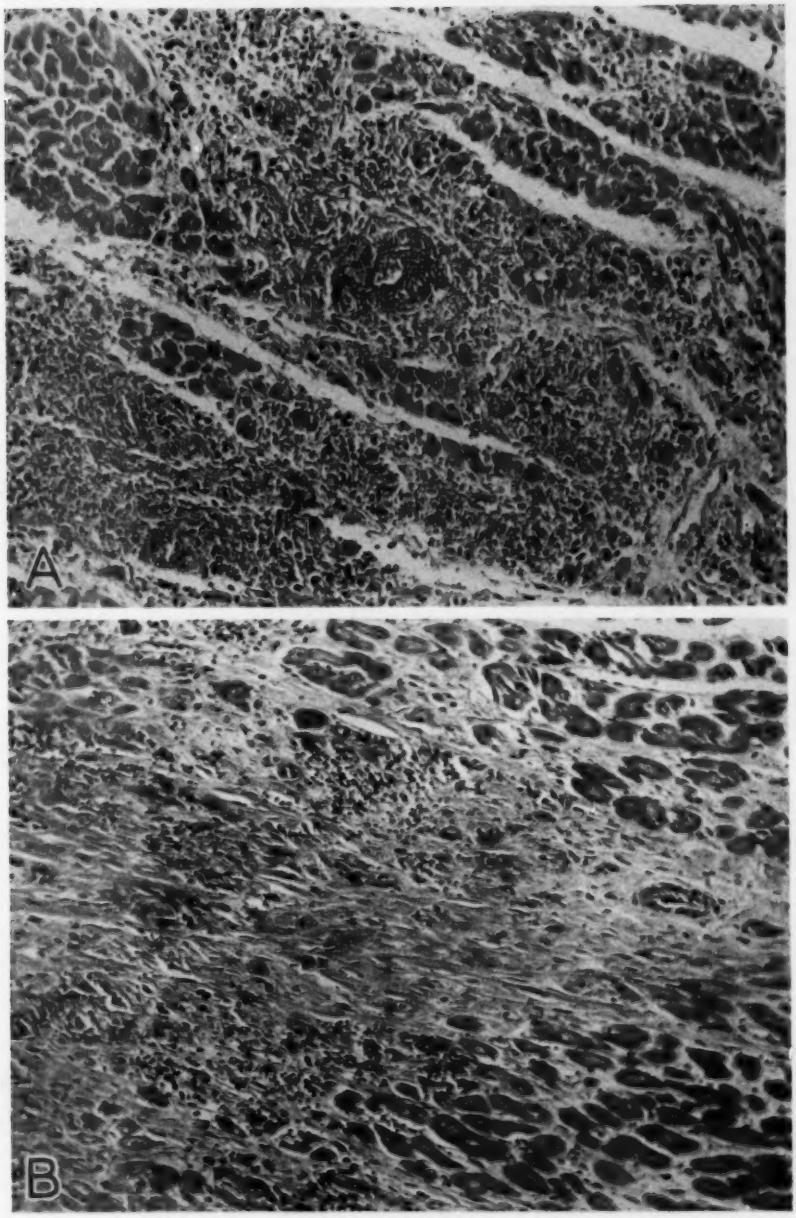

Fig. 8.—Lesions of the myocardium as observed in a nephrectomized dog:

A (same dog as in fig. 1 *A*), subacute lesion showing necrotic myocardium replaced by granulation tissue. Note the necrotic arteriole near the center of the figure. × 139.5.

B (same dog as in fig. 1 *A*), hyaline scar of the myocardium containing a few lymphocytes, hemosiderin granules and capillary remnants. × 139.5.

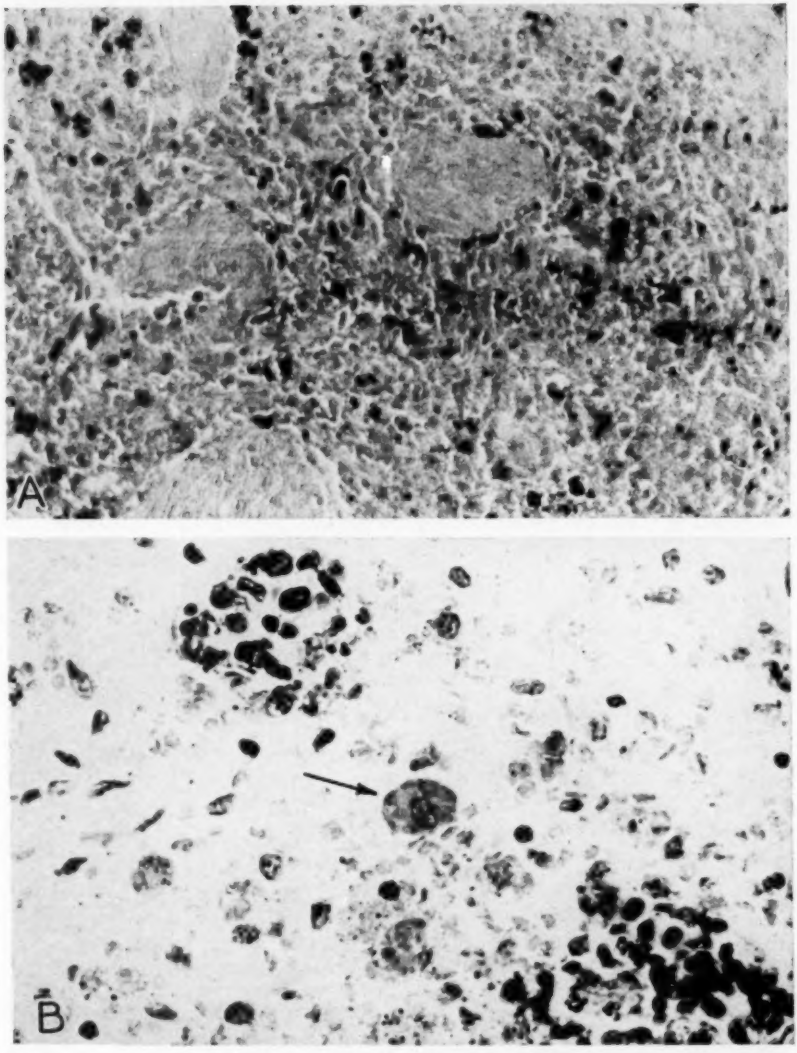

Fig. 9.—Microscopic appearance of lymph node and spleen of bilaterally nephrectomized dogs:

A (same dog as in fig. 5*A*), marked hemosiderosis of the spleen. The dark granules represent hemosiderin deposits as revealed by iron stain. × 232.

B (same dog as in fig. 2*A*), macrophage in the medullary sinus of a lymph node, showing marked erythrophagocytosis; the cytoplasm contains several red blood cells. × 752.

Whereas in our previous reports,[6] dealing with changes in various tissues following bilateral nephrectomy, the longest survival was 19½ days and the over-all mean survival was eight days, in the present series of 22 dogs, 63 per cent survived over 10 days after nephrectomy (27 per cent for 10 to 20 days and 36 per cent for 23 to 70 days). All these animals had persistent hypertension after the first week following nephrectomy, with values varying between 145 and 205 mm. Hg and an average of 173 mm. Hg. The hemodynamics of the circulation in these animals was characteristic of that observed in hypertension induced by partial constriction of the renal artery or by constriction of the renal parenchyma and in hypertensive cardiovascular disease as it occurs in the human.[7]

Of the 14 dogs surviving 10 days or more without kidneys, only one, which survived 19 days, failed to reveal cardiovascular lesions on gross inspection and random sampling of tissues for microscopic study (table 3). Necrosis of the heart was common in the entire group, but fibrosis of necrotic foci was commonly observed only in animals surviving 20 or more days. Although necrosis of arteries and arterioles with smudging was noted sporadically in the entire group, the

TABLE 3.—*Incidence of Cardiovascular Lesions and Their Correlation with the Type of Operation (Nephrectomy or Ureteral Ligation), Period of Survival and Mean Blood Pressure*

			Arteries		Heart			Mean Blood Pressure, Mm. Hg		
Operation	Animals	Period of Survival, Days	Necrosis (Hyperacute and Acute)	Hyaline (Subacute and Chronic)	Necrosis (Acute)	Scars (Subacute and Chronic)	Animals Devoid of Lesions	150 or Less	151-160	Over 160
Nephrectomy.....	4	< 6	0	0	0	0	4	3	0	0
Nephrectomy.....	5	6 to 10	0	1	3	2	2	4	0	1
Nephrectomy.....	5	11 to 20	2	0	4	2	1	0	0	5
Nephrectomy.....	8	>21	4	7	6	5	0	0	2	6
Ureteral ligation....	2	<10	1	0	1	0	1	1	0	0
Ureteral ligation....	5	10 to 20	1	1	2	0	3	4	0	0
Ureteral ligation....	3	>20	1	0	1	0	2	3	0	0

arterial-arteriolar lesions varied in appearance. In animals with the more prolonged periods of survival, the medial necrosis was most often of the acute or early hyaline type, that is, with hyalinization of the necrotic media, with or without nuclear remnants persisting. In addition, the animals surviving more than 20 days displayed the subacute and chronic arterial-arteriolar lesions, that is, subendothelial fibrosis or hyalinization with atrophy of the media, thickening of the internal elastic lamina, fibrous replacement of the vessel wall and total hyalinization of the vessel wall (table 3).

The group with bilateral ureteral ligation, 80 per cent of which survived more than 10 days (50 per cent for 10 to 20 days, 30 per cent for 21 to 30 days), had a mean arterial blood pressure after the first week that varied between 100 and 140 mm. Hg with an average of 126 mm. Hg. Five of the eight animals surviving more than 10 days had no hypertension, and the other three had only a mild degree of elevation of the arterial pressure. Moreover, the cardiovascular lesions were less frequent in this group. Six of the 10 animals showed no cardiovascular lesions on gross inspection and random sampling of the tissues. The four animals showing

7. Grollman, A.; Turner, L. B.; Levitch, M., and Hill, D.: Am. J. Physiol., to be published.

lesions displayed almost entirely hyperacute and acute lesions, that is, necrosis of the heart without evidence of repair, and necrosis of arteries and arterioles with smudging (table 3).

COMMENT

The present series of dogs subjected to bilateral nephrectomy have demonstrated not only lesions similar to those previously described [6] but, in addition, other characteristic cardiovascular lesions. The cardiac lesions, which in the earlier group consisted mainly of necrosis, have passed after 10 days into the reparative phase. Granulation tissue was evident particularly in dogs surviving 10 to 20 days. Thereafter, fibrosis and the formation of hyaline scars appeared in the myocardium. Previously it was pointed out that the necrosis observed in the heart appeared, at least in part, to be independent of the vascular lesions but in the present study this conclusion could not be substantiated.

The vascular lesions can be divided into three distinct categories. The first group consists of necrosis and smudging or smearing of the smooth muscle of the media of small arteries and arterioles. Since this lesion is fulminant and can be observed a few days after bilateral nephrectomy, it has been termed a hyperacute lesion. The second lesion is characterized by necrosis of the media with a tendency toward hyalinization, without the explosive nature of the hyperacute lesions. Within the media, remnants of nuclei are evident. The nature of this lesion, particularly the retention of the shape of the media, and the pyknosis or karyorrhexis, suggests a lesion of slower development than the hyperacute type. Moreover, since it does not become apparent until after 10 to 20 days, it has been designated as an acute arterial-arteriolar lesion. The third category is characterized by a series of vascular changes involving small arteries and arterioles. Usually, when one or more of these series of changes occurs in an artery or an arteriole, the lumen becomes smaller and frequently eccentric. The entire wall may become hyalinized and appear fixed. The subendothelial zone may show an accumulation of connective tissue or of a hyaline substance or a mixture of these two. The internal elastic lamina may become thickened. These three changes are associated with atrophy and disappearance of the smooth muscle of the media. On occasions an irregular endothelial proliferation occurs. Finally the entire vessel wall may become thickened, owing to increased cellularity which seems to represent connective or fibrous tissue replacement of all layers. These changes of the third category were observed when survival had exceeded 20 days and have, therefore, been designated as subacute and chronic. The change in the diameter of the lumen of the small arteries and arterioles apparently results from the laying down of materials in the vessel wall, either in the form of a hyaline substance or in that of connective tissue elements and, if sufficiently widespread, interferes appreciably with the circulation. The subacute and chronic lesions, which occurred only in the animals surviving longer than 20 days, were accompanied to a varying degree by the hyperacute and acute lesions observed in animals surviving for shorter periods.

It is not possible, as pointed out by Moritz and Oldt,[8] to distinguish absolutely between small arteries and arterioles. The lesions described in the present paper and those seen in the human appear to involve mainly the terminal portions of the

8. Moritz, A. R., and Oldt, M. R.: Am. J. Path. **13**:679, 1937.

arterial tree. Although a diameter of 100 microns has been defined as the dividing point between arteries and arterioles, vessels well over this diameter were involved in the same manner as those below this size.

The hyperacute and acute lesions which we have observed are similar to those described by several other workers in experimental hypertension.[9] The subacute and chronic lesions, however, have characteristics similar to the arteriolar lesions described by Moritz and Oldt [8] in the human. Certain of these lesions resemble the arteriolar sclerosis described by Goldblatt [9c] in experimental hypertension in the dog.

The relatively short survival of bilaterally nephrectomized dogs subjected to the artificial kidney was attributed previously [1b] to the ravaging affects of the hyperacute and acute cardiovascular lesions. It is apparent from the present study that the cardiovascular lesions can be modified and that the survival can be extended to a considerable degree. A major factor contributing to this extension of survival appears to be the marked improvement in the technic of maintaining renal excretory function [3] and the sodium restriction to which these animals were subjected. Peritoneal irrigation not only is a simpler and more effective procedure for removing metabolic waste products but also maintains a more normal water and electrolyte balance.[3] Although loss of weight occurred, and a rare animal with partial intestinal obstruction became emaciated,[3] most of the animals have been maintained in good condition. Periodic blood transfusions have maintained a satisfactory red blood cell level. The antibiotics added to the irrigating fluids have prevented infection, as demonstrated by the low incidence of bronchopneumonia or acute peritonitis.[3] With extended survival, the cardiovascular lesions following bilateral nephrectomy have accordingly been modified to resemble those associated with chronic hypertension in the human. The level of the blood pressure, per se, however, is not the only factor responsible for the development of the subacute and chronic forms of vascular lesions, since comparable elevations of blood pressure when induced by constriction of the renal artery or by figure of eight ligature of the kidney with contralateral nephrectomy give rise to much less pronounced lesions.[9c]

The observations on the dogs subjected to ureteral ligation throw further light on the genesis of the vascular lesions. Although eight of these animals survived more than 10 days, five displayed no evident cardiovascular lesions, and the remaining three manifested only the hyperacute and acute varieties. Elevation of blood pressure was either absent or negligible except during the first week following operation.[1a] The presence of renal tissue, accordingly, offers protection against the development of hypertension and cardiovascular lesions. The fact that in some animals after ureteral ligation hyperacute lesions develop in the absence of hypertension tends to indicate a separate background for these two processes.[2] This modification of the tissue changes associated with the presence of renal tissue is further evidence for the view that the kidney exerts an action other than its excretory function and that this action is involved with the maintenance of the normotensive state.[10]

9. (*a*) Winternitz, M. C., and Waters, L. L.: Yale J. Biol. & Med. **12**:451, 1940. (*b*) Wilson, C., and Pickering, G. W.: Clin. Sc. **3**:343, 1938. (*c*) Goldblatt, H.: The Renal Origin of Hypertension (American Lecture Series, no. 14, American Lectures in Pathology), Springfield, Ill., Charles C Thomas, Publisher, 1948, pp. 29-36.

10. Grollman, A., in Bell, E. T.: Symposium on Hypertension, Minneapolis, University of Minnesota Press, 1951.

Counterparts of the lesions observed in the present study may be seen in human tissues from persons suffering with hypertensive cardiovascular disease. The hyperacute and acute vascular lesions occur predominately in the malignant (rapidly advancing) form of human hypertension, whereas the subacute and chronic forms occur particularly in the so-called benign form of hypertension. In addition to hypertension, bilaterally nephrectomized dogs also show a profound anemia, which will be described in a separate communication.[11] The combination of hypertension, cardiovascular lesions and anemia is similar to the disturbance observed in human hypertension, especially that of the rapidly advancing variety. The hemodynamics of the circulation of the nephrectomized dog has also been demonstrated to be identical to that characteristic of hypertensive cardiovascular disease as observed in the human.[7] Hence it appears that the hypertension of the human is similar to that observed following bilateral nephrectomy in dogs. This must be taken into account in any theory purporting to explain the pathogenesis of experimental and clinical hypertension.

SUMMARY

Tissues of dogs subjected to bilateral nephrectomy or ureteral ligation and maintained alive for periods up to 70 days were studied.

The extended survival of these animals was associated with changes in the characteristics of the cardiovascular lesions previously encountered with survival up to 10 days. The previously described lesions of the heart showed the necrotic foci being replaced by granulation tissue or hyaline scars. The small arteries and arterioles, in addition to the previously described necrotic lesions, revealed also a form of necrosis of the media consisting of early hyalinization with pyknosis and karyorrhexis and a series of changes including total hyalinization, fibrous replacement of the vessel wall ("hyperplasia"), possible hypertrophy of the media of precapillary arterioles, subendothelial hyaline deposits, subendothelial fibrosis of the endarteritic type, atrophy of the media, thickening of the internal elastic lamina and endothelial proliferation. The fulminant arterial-arteriolar necrosis has been termed a hyperacute lesion; the early hyalinization of the media with or without nuclear remnants has been termed an acute lesion, and the other changes have been considered as subacute or chronic lesions. It is our opinion that all these lesions are comparable to those observed in the tissues of humans in the hypertensive state.

The extended survival of these dogs was accompanied by an elevation of blood pressure to hypertensive levels as previously described in animals surviving for shorter periods. Since the same magnitude of hypertension occurred in both groups, the different cardiovascular lesions noted are apparently dependent on factors other than the blood pressure level.

Although bilateral ureteral ligation did not prevent the development of the hyperacute cardiovascular lesions, the presence of intact renal tissue was associated with either no or a minimal elevation of blood pressure and with an absence of the "chronic" type of cardiovascular lesions.

Any theory attempting to explain the pathogenesis of hypertension must take into account the fact that hypertension and arteriolar sclerosis occur experimentally in the absence of renal tissue and that the presence of renal tissue appears to be protective against these two disturbances.

11. Muirhead, E. E.; Jones, F., and Grollman, A.: To be published.

IN VITRO EFFECTS OF CORTISONE ON MESODERMAL TISSUE

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THE ADMINISTRATION of adrenocorticotropic and adrenocortical substances to humans results in a transient slight rise in the number of circulating lymphocytes and eosinophilic leukocytes followed by a marked, more prolonged depression.[1] This is believed to be an effect of the adrenocortical 11,17-oxysteroids. The decline of circulating eosinophils has been described as a practical laboratory test for determining the physiologic effects of the pituitary adrenocorticotropic factor, called ACTH, and cortisone (11-dehydro-17-hydroxy-corticosterone).[2] The mechanism through which this eosinopenia and lymphopenia result has not been clarified.

Cortisone has been shown to delay the healing of experimental wounds.[3] Histologic examination of these lesions demonstrates the failure of connective tissue elements, particularly fibroblasts, to initiate the healing process. The manner in which this inhibition of fibroblastic activity is brought about is unknown.

The present study was undertaken to determine whether the above effects are produced by cortisone acting directly on the cells involved.

METHODS AND MATERIALS

Culture of human leukocytes was the first method of study. The slide technique was used throughout.[4] Blood was obtained by venipuncture from seven normal individuals. The buffy coat was separated by immediate low speed (1,800 rpm) centrifugation. The intact coat was

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The cortisone acetate used in this study was supplied by Dr. J. M. Carlisle, medical director of Merck & Company, Inc., Rahway, N. J.

From the Department of Dermatology and Syphilology, University of Pennsylvania School of Medicine (Donald M. Pillsbury, M.D., director).

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3. Ragan, C.; Howes, E. L.; Plotz, C. M.; Meyer, K.; Blunt, J. W., and Lattes, R.: Bull. New York Acad. Med. **26**:251-254, 1950.

4. Cameron, G.: Tissue Culture Technique, ed. 2, New York, Academic Press, Inc., 1950.

removed from the clot and minced into pieces measuring about 1 mm. in diameter. Each of these fragments was explanted onto a sterile cover slip. One drop each of chick plasma, human serum and chick embryo extract was added to each explant. In the test preparations the human serum contained 50 micrograms of cortisone acetate (no preservative) per milliliter. After sealing, all cultures were incubated at 37.5 C. About 20 preparations were made for each individual. Measurements of the extent of leukocyte migration were recorded at four and 17 hours. Wright's stained preparations were made at intervals for differential leukocyte counts in the migratory zone. In a manner similar to that just described, explants from chick embryo heart and thigh muscle were studied for fibroblast proliferation in the presence of cortisone. Observations were made at 12-hour intervals on more than 100 tissue cultures.

In the second part of the experiment, heparinized specimens of the peripheral blood of six normal humans were used as the test material. Four-tenths milliliter samples of these blood specimens were pipetted to clean, dry test tubes. To each of these tubes was added 0.1 ml. of 0.1 per cent cortisone acetate solution or 0.01 per cent cortisone acetate solution. The cortisone acetate used in these experiments was made up in an original stock solution of 95 per cent alcohol. Tenfold dilutions in isotonic sodium chloride solution were made up so that the 0.1 per cent cortisone acetate solution contained 0.95 per cent alcohol. The controls consisted of isotonic sodium chloride solution only and of saline solution containing amounts of alcohol corresponding

TABLE 1.—*Differential Leukocyte Counts on Tissue Cultures Incubated with Cortisone in Vitro* *

Period of Incubation	Tissue Culture Preparation or Control	Subject 1				Subject 2				Subject 3				Subject 4			
		P†	M	L	E	P	M	L	E	P	M	L	E	P	M	L	E
2 hr.	Saline control	41	19	39	1	54	3	43	0	63	10	21	6	48	8	20	24
	0.1% cortisone	47	39	9	4	58	9	33	0	52	10	20	18	59	13	2	23
	0.1% alcohol control	54	11	33	2	62	6	32	0	56	4	22	18	41	21	7	30
	0.01% cortisone	34	8	57	1	60	2	37	1	63	3	28	7	54	19	10	25
	0.01% alcohol control	48	16	30	5	58	3	39	0	62	7	23	8	36	13	30	21
17 hr.	Saline control	20	19	55	6	..	..	..	..	36	3	51	10	28	2	47	23
	0.1% cortisone	30	17	50	3	..	..	..	..	28	4	43	25	24	1	45	29
	0.1% alcohol control	51	5	43	1	..	..	..	..	59	2	38	19	..	..	..	..
	0.01% cortisone	24	22	49	1	..	..	..	..	30	0	53	17	18	3	55	24
	0.01% alcohol control	41	21	35	3	..	..	..	..	60	4	25	11	26	4	42	27

* Each set of figures represents 100 cells counted.

† Key: P stands for polymorphonuclear leukocytes; M, monocytes; L, lymphocytes; E, eosinophilic leukocytes.

to the test preparations. Blood smears and eosinophil chamber preparations from each tube were made immediately and after the tubes had been incubated for two and 17 hours at 37.5 C. The blood smears were stained and 100 cell differential counts performed. The eosinophil chamber counts were done by the method described by Thorn and associates.[2]

RESULTS

The tissue culture studies of the human buffy coats showed no inhibition of migration of any cellular elements in contact with any concentration of cortisone. Differential counts made in the peripheral, actively migrating zone were considered equivocal because of the large variance between different areas of the same cultures.

Chick embryo fibroblast proliferation was profuse in the presence of cortisone, and there was no inhibition of activity as compared with the saline controls. No cytologic alterations were demonstrable in the preparations exposed to the drug.

Blood smears made after blood had been incubated with different concentrations of cortisone for two and 17 hours showed no quantitative changes as compared with the controls (table 1). There was an apparent increase in the percentage of lymphocytes and eosinophils with a decrease in the percentage of polymorphonu-

clear leukocytes and monocytes in both controls and test preparations. This is probably referable to the varying degrees of natural resistance to lysis of these different types of cells following prolonged incubation.

Eosinophil chamber counts showed no decrease in this element following prolonged incubation with cortisone, as compared with the controls (table 2).

COMMENT

The possibility that the eosinopenia and lymphopenia resulting from the action of certain corticoids might be direct cytoxic conditions was suggested by the recent studies of Dougherty and White.[5] They found a dissolution of medium size and small lymphocytes within mouse lymphoid tissue one to six hours after the injection of ACTH. This effect was not observed in adrenalectomized animals.

Heilman [6] in tissue culture studies of lymph node explants grown in contact with cortisone found moderate decreases in the numbers of small lymphocytes in the migrating zone. After examination of a large number of stained preparations of this type, we concluded that differential counts of this type must be evaluated

TABLE 2.—*Eosinophil Chamber Counts After Blood Cells Had Been Incubated with Cortisone in Vitro*

Subject	Immediate Baseline Count on Saline Suspension	Counts Made After 2 Hours' Incubation				
		Saline Control	0.1% Cortisone	Alcohol Control	0.01% Cortisone	Alcohol Control
1	175 *	75	81	88	75	88
2	164	175	164	181	188	188
3	100	94	112	131	112	81
4	94	75	75	75	100	131
5	295	225	288	225	200	269

* Each figure represents an average of four counts expressed in number of cells per cubic millimeter.

with caution because of the large discrepancies in counts made from different parts of a single culture.

The same author [7] has studied the effects of cortisone on fibroblast preparations similar to the ones reported here. She felt that some inhibition of proliferation could be demonstrated at dose levels of about 50 micrograms per milliliter. A possible explanation of the divergence between Heilman's results and ours may lie in the possible inclusion of contaminant material in the less pure preparations in use at the time her studies were performed.

The in vitro studies of the effects of corticoids on suspensions of lymphocytes from experimental animal tissues have been conflicting. Robertson [8] could demonstrate no lysis of rat lymphocytes incubated for long periods with an extract of the adrenal cortex (eschatin[8]). Schreck,[9] on the other hand, using a method of intravital staining, found decreased viability of thymic lymphocytes suspended in purified adrenal cortical extracts including cortisone. Hechter and Stone [10] found that

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6. Heilman, D. H.: Proc. Staff Meet., Mayo Clin. **20**:310-312, 1945.
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8. Robertson, J. S.: Nature, London **161**:814, 1948.
9. Schreck, R.: Endocrinology **45**:317-334, 1949.
10. Hechter, O., and Stone, D.: Federation Proc. **7**:52, 1948.

it was necessary to add homogenates of lymphoid tissues to lymphocyte suspensions to achieve any appreciable degree of lysis with corticoids.

From the data presented here it is apparent that cortisone has no direct in vitro effect on the viability of circulating human leukocytes and that proliferation of the embryonic fibroblast is not inhibited by the drug. This means that the well established in vivo eosinopenic and lymphopenic action of the corticoids, as well as their depressant activity in wound healing, must be explained by some different mechanism or by interaction of corticoids and a substance not present in our preparations.

CONCLUSIONS

Under the conditions of these experiments cortisone alone displays no in vitro cytotoxic activity against human peripheral leukocytes.

Proliferation of chick embryo fibroblasts is not inhibited in tissue cultures containing cortisone.

SERUM GLYCOPROTEINS IN EXPERIMENTAL SCURVY

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SERUM glycoproteins relate to proteins having low nitrogen and high carbohydrate content.[1] Such carbohydrate (polysaccharide)-containing proteins have been isolated from normal and pathological serums.[2] That similar substances are present in tissue had been inferred from histochemical observations and from the fact that certain polysaccharides have been isolated from tissues.

Considerations advanced in recent papers[3] have indicated that the level of circulating glycoproteins might be a reflection of the state of the ground substance of the connective tissue. It was proposed that depolymerization of glycoprotein constituents of the ground substance would lead to an increase of their water solubility and to an increase of their concentration in the blood serum.

Ascorbic acid (vitamin C) deficiency produces, in scurvy, a disease characterized by general involvement of the intercellular matrix of mesenchymal tissues.[4] Salient manifestations of this condition are hemorrhages, weakness, and signs and symptoms related to articular and skeletal lesions. Indications of a depolymerization occurring in the connective tissue of scorbutic guinea pigs were obtained in histochemical studies.[3] The objective of the present study was to determine whether such animals also show the corollary increase of serum glycoproteins.

The opinions expressed in this paper are those of the authors and do not necessarily represent the official views of any governmental agency.

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From the Army Medical Nutrition Laboratory (Dr. Pirani) and the Department of Pathology, University of Illinois College of Medicine (Drs. Pirani and Catchpole).

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METHODS

Young adult male guinea pigs, about 8 weeks of age and weighing 350 to 400 Gm., were obtained from a local large distributor. The animals were placed in single wire screen-bottom cages and adjusted to laboratory conditions for about a week. All guinea pigs were fed an ascorbic acid-free diet [5] and given tap water ad libitum. Four groups of animals were studied: (1) controls, receiving a supplement of 2.5 mg. of ascorbic acid per day orally or adequate greens (lettuce); (2) guinea pigs in which "chronic scurvy" had been produced, receiving 0.2 mg. of ascorbic acid per day; (3) guinea pigs in which "acute scurvy" had been produced, receiving no ascorbic acid; (4) a "rehabilitation" group, a small number of animals rendered scorbutic and then fed a diet adequately fortified with ascorbic acid for seven days. Guinea pigs pair-fed with animals in groups 2 and 3 and reciving 2.5 mg. of ascorbic acid per day, were available as additional controls. When the requisite clinical picture of acute or chronic scurvy

TABLE 1.—*Experimental Data*

Animal Group	Treatment	Animals	Time on Diets, Days	Av. Daily Wt. Gain or Loss, Gm.	Scorbutic Lesions at Autopsy
1	Acute scurvy	17	19-35	−4.9	++ to +++
2	Controls: pair-fed with animals of acute scurvy group	8	20-33	−2.4	−
3	Controls: fed ad libitum	5	20-32	+3.5	−
4	Chronic scurvy	8	21-33	+0.6	++ to +++
5	Controls: pair-fed with animals of chronic scurvy group	6	21-33	+2.4	−
6	(a) Acute scurvy	6	20	−2.0	++ to +++
	(b) 5 animals of group 6a rehabilitated with 5 mg. of ascorbic acid per day	5	7	+4.2	Clinical improvement but lesions still obvious

TABLE 2.—*Glycoprotein Levels*

Group	Treatment	Animals	Glycoprotein Determined as: Galactose-Mannose, Mg./100 Cc.	Glycoprotein Determined as: Tyrosine, Mg./100 Cc.
1	Acute scurvy	13	67.2 ± 21.6 *	6.2 ± 2.0
2	Chronic scurvy	7	51.9 ± 16.2	7.8 ± 2.1
3	Rehabilitation	5	40.2 ± 4.8	5.5 ± 0.5
4	Controls	19	27.9 ± 6.2	4.4 ± 0.8

Groups	Group Intercomparisons on Basis of: Carbohydrate	Tyrosine
1 and 4	P † < 0.01	P < 0.01
2 and 4	P < 0.01	P < 0.01
3 and 4	P < 0.01	P < 0.01

* The ± value is the standard deviation.
† P was calculated by Fisher's "t" test.

had been obtained, the animals were anesthetized with pentobarbital sodium (nembutal® sodium), administered intraperitoneally (3 mg. per 100 Gm. of body weight), and bled from the right side of the heart. Blood was allowed to clot and the serum separated. Complete autopsies were then performed. The serum specimens were analyzed for glycoproteins by the methods of Winzler and associates [2b]: (1) Sugar was determined with the orcinol reagent against a standard of galactose-mannose; (2) tyrosine was determined with the Folin-Ciocalteu reagent.[6]

RESULTS

Experimental data concerning the various experimental animals are listed in table 1. Glycoprotein levels as reflected by sugar and tyrosine determinations appear in table 2, with pertinent statistical details.

5. Rockland® guinea pig diet especially prepared by the Arcady Mills Company of Chicago.

6. Folin, O., and Ciocalteu, V.: Tyrosine and Tryptophane Determinations in Proteins, J. Biol. Chem. **73**:627, 1927.

The factor of inanition was partially controlled by the use of controls pair-fed with the deficient animals. Animals completely deprived of ascorbic acid lost weight and their pair-fed controls also, though to a lesser extent. Animals in the chronic scorbutic category held their weight, or gained slightly, and their controls gained to a larger extent. Whether gaining or losing weight, the pair-fed controls did not differ in serum glycoprotein levels from the ad libitum-fed controls. These animals were therefore treated as a single group. It is noteworthy also that animals in the rehabilitation group, which showed the greatest daily gains of weight, continued to exhibit enhanced amounts of serum glycoproteins.

Two controls and one scorbutic animal had pneumonia at autopsy. In keeping with the findings of Winzler and associates [2b] and Seibert and associates,[7] the serum glycoprotein levels of these three animals were abnormally high. They were eliminated from the present study. Both the guinea pigs with acute and those with chronic scurvy showed highly significant increases in serum glycoproteins compared with normal guinea pigs. The differences between the glycoprotein values of the two scorbutic groups were not significant. Clinically these animals showed stiffening of joints and hemorrhages, with loss of weight in the group with acute scurvy and practically no gain in the group with chronic scurvy. At autopsy the severity of scurvy was evaluated and graded (1 + to 3 +) on the basis of enlargement of the joints, costochondral beading and extent of hemorrhage in the same areas. Previously this had been found [8] to correlate well with the severity of the histological lesions and was therefore considered to be an adequate criterion of the severity of the disease for the purpose of this study. However, there did not appear to be a close correspondence between the estimated severity of the disease and the glycoprotein levels. In the "rehabilitation" group there was clinical improvement in some but not in all animals, while serum glycoprotein values fell somewhat. At autopsy the lesions of scurvy, although less prominent, were still obvious.

COMMENT

The earlier experiments of Wolbach and Howe [4b] had suggested that with complete deprivation of vitamin C certain skeletal tissues formed a fluid material rather than dentin or bone. Following administration of the vitamin, rapid solidification or "jellation" of this fluid occurred. On the basis of histochemical studies,[3a] the process in scurvy was indicated to be a depolymerization of the ground substance of connective tissue. Work on the depolymerizing reaction indicated that the increased serum mucoproteins shown to appear in certain diseases and following experimental procedures (trauma, infections, tumors) could have as their source this altered connective tissue.[3b] On this basis, the finding of elevated serum glycoproteins in acute and chronic scurvy of guinea pigs was not unexpected, and lends support to the concept of a depolymerized ground substance as a feature of the disease.

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Rise of serum glycoproteins has been shown to follow several "unspecific" stimuli, some of which have been controlled in this study. Thus, the serum glycoproteins rise in fever; however, it is characteristic of scorbutic guinea pigs to have rectal temperatures lower than normal.[9] Wasting of tissues, which might contribute to raised serum glycoprotein levels, was partially compensated by the use of pair-fed controls. These animals showed normal levels of glycoproteins. The possibility that necrosis per se contributes to the glycoprotein pool is indicated by recent studies.[10] However, it is scarcely possible to regard necrosis or a more general "tissue destruction" as a unitary process. In scorbutic guinea pigs, for example, necrosis is limited almost exclusively to tissues of mesenchymal origin, and it is clearly impossible here to separate the final picture of necrosis from earlier manifestations of connective tissue breakdown.

The elevated serum levels of glycoprotein, taken as an indication of depolymerization of the ground substance, could be the result of two separate phenomena which could occur either separately or in combination: Either glycoproteins produced by fibroblasts fail to become polymerized in the absence of ascorbic acid, or glycoproteins of normally formed ground substance undergo depolymerization. Results of histological and histochemical studies,[11] although not conclusive, favor the concept that both factors are at play in determining high serum glycoprotein levels. The present findings raise the question whether in the scorbutic state there is a failure of formation of extracellular material, as shown histologically by numerous investigators. It is possible that in scurvy extracellular material is produced, but because of its depolymerized state, leading to low viscosity and water solubility, it rapidly enters the blood stream and fails to be deposited about connective tissue cells as in normal animals. At any rate, complete cessation of the production of ground substance probably occurs only in the very advanced scorbutic state, while depolymerization of the ground substance would appear to develop much earlier in the disease.

The present findings have confirmed the belief that serum glycoproteins increase in infectious diseases in guinea pigs, whether these are affected with scurvy or not. High levels of serum glycoproteins have been demonstrated in tumor-bearing mice,[3b] a species not susceptible to scurvy. It therefore appears that enzymes and hormones which provoke depolymerization of the ground substance of connective tissue may do so in the presence of an adequate intake or synthesis of ascorbic acid. Nevertheless, the possibility that systemic or local ascorbic acid deficiency is an etiologic factor in other states involving connective tissue change should not be

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10. Schlamowitz, S. T.; DeGraff, A. C., and Schubert, M.: Studies of Tissue Response to Injections of Enzymes, Circulation **1**:822, 1950. Simkin, B.; Bergman, H., and Prinzmetal, M.: Studies on Coronary Circulation: V. Quantitative Changes in a Serum Mucoprotein Following the Occurrence of Myocardial Infarction, Am. J. Med. **6**:734, 1949.

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overlooked. The fact that the levels of serum glycoproteins are high in scurvy raises the question whether they are involved in the genesis of amyloid reported in this disease.[12]

SUMMARY

The level of serum glycoproteins is significantly elevated in acute and chronic scurvy of guinea pigs. Rehabilitation with ascorbic acid reduced the amount of circulating glycoproteins, although normal levels were not reached in the experimental period employed. High serum polysaccharide values are consonant with the theory that scurvy involves a depolymerization of carbohydrate-containing constituents of the ground substance of connective tissue. These smaller, water-soluble entities are thereupon released into the circulation. The possibility that this process is related to the formation of amyloid is being studied.

Technical assistance was given by Mr. John D. Taylor and Sgt. Paul J. Weber.

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ATTEMPTS TO PRODUCE RHEUMATIC CARDITIS IN LABORATORY ANIMALS BY MEANS OF STREPTOCOCCIC INJURY

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SINCE clinical and epidemiologic evidence indicates an association of streptococcal infection and the onset of rheumatic fever,[1] numerous attempts have been made to produce rheumatic fever experimentally by means of streptococcic infection in animals. Topley and Wilson[2] have succinctly reviewed the role of streptococci in rheumatic diseases. This may be summarized in the observation that any streptococcus of sufficient virulence readily causes carditis and arthritis in rabbits but that significant pathologic and clinical differences distinguish such experimental diseases from those in rheumatic patients.

Many infectious micro-organisms have been suspected in the etiology of rheumatic fever, but none have been definitely implicated by bacteriologic isolation and the fulfilment of Koch's postulates. Extensive cultural studies have not disclosed any significant presence of infectious agents in rheumatic lesions.[3] This has led to emphasis of the host factor in rheumatic fever and to popular acceptance of an allergic hypothesis of the genesis of rheumatic fever. However, allergic diseases such as serum sickness or various atopies also differ anatomically and clinically from human rheumatic diseases.

More recently Swift and co-workers[4] have considered the factor of multiple type group A streptococcal infection to be of paramount importance in the causation of rheumatic fever. Some aspects of this factor were studied during these experiments.

The most definitive criterion for rheumatic fever is the typical Aschoff body. This has never been observed except in active human rheumatic fever. Some workers have considered somewhat ill defined experimental lesions to be the animal counterpart to human rheumatic fever. At present it does not seem permissible to accept such a view, and instead the strict criterion of a typical Aschoff body should be retained. While these studies did not produce typical Aschoff bodies, and hence

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The opinions expressed herein are those of the author and cannot be construed as reflecting the views of the Navy Department or of the Naval Service at large. Endorsement of commercially available materials is not implied by their usage in this connection.

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3. Angevine, D. M.; Rothbard, S., and Cecil, R. L.: Cultural Studies on Rheumatoid Arthritis and Rheumatic Fever, J. A. M. A. **115**:212 (Dec. 14) 1940. Wilson and Miles.[2]

4. Murphy, G. E., and Swift, H. F.: J. Exper. Med. **89**:687, 1949. Swift.[1]

no claims for the production of rheumatic fever can be made, closer approximations to this goal were attained and have engendered the hope that eventually success may be possible.

These studies were primarily directed to the reproduction of rheumatic fever in rabbits and Macacus mulatta monkeys. No attempt was made to delve into the complex relationships between the host and the streptococci, or the elucidation of the mechanisms of streptococcal injury. Part 1 of these experiments consisted of preliminary, screening studies designed to indicate methods most likely to succeed. Part 2 subsequently describes more quantitative evaluations of the carditis associated with streptococcal injury in rabbits.

METHODS

Part 1.—It was previously found [5] that when sterile streptococcus NY-5 (group A, type 10) filtrate was injected subcutaneously into rabbits daily for several weeks an extensive carditis developed, somewhat resembling that observed in human rheumatic fever. Preliminary studies were made, extending such observations in rabbits and rhesus monkeys. They consisted in studying various methods of preparing, storing, and administering toxic filtrates.

The following nontoxic medium was found successful for the production of streptococcal filtrate:

Disodium phosphate, anhydrous, C. P.	10.6 Gm.
0.1 molar citric acid, C. P.	25.4 cc.
Dextrose, C. P.	2.0 Gm.
Magnesium sulfate U. S. P. (Epsom salt)	2.0 Gm.
Potassium nitrate, C. P.	1.0 Gm.
Sodium chloride, C. P.	5.0 Gm.
Liver extract, crude (Lilly)	5.0 Gm.
Distilled water	1,800 cc.

Five per cent defibrinated rabbit blood was added to this isotonic, pH 7.6 buffered medium sterilized by Seitz filtration. A 1 per cent inoculum of a 16-hour Difco brain-heart infusion broth culture of the streptococcus was employed. Filtrates of the NY-5 and the 98 (group A, untypable) streptococcus strains were used. Incubation at 37 C. with occasional swirling was continued until two hours past the time of complete hemolysis. Smears and cultures were made to check purity of growth; the cultures were centrifuged for 40 minutes at 2,400 rpm. and 1 C., and the supernatant was filtered with vacuum through a 14 cm. Seitz filter at 2 C. The filtrate was tested for sterility and frozen in solid carbon dioxide in half-filled screw-capped bottles. The filtrates had a pH of 7.2 to 7.4, and did not completely hemolyze 0.5 cc. of a 2 per cent suspension of washed rabbit red cells at 37 C. in two hours in amounts of 1 cc. or less.

Injections of filtrate were varied as to dose and route to achieve moderate illness extending over several days or weeks. In a group of 56 young albino rabbits and 7 monkeys filtrate was injected alone. In a group of 41 rabbits and 21 monkeys filtrate was injected combined with viable alpha and the NY-5 or 98 strains of beta hemolytic streptococci. A group of four monkeys were given intranasally 1 cc. amounts of 16-hour Difco brain-heart infusion broth cultures of the NY-5 and 98 streptococci twice weekly for six weeks. A similar group of four were given intranasal doses of these cultures with additional intramuscular injections of 5 cc. of filtrate per kilogram of body weight.

Various minor trials were made of NY-5 or 98 streptococcal serum extracts prepared by a modified method of Weld.[6] These contained considerable streptolysin S, as shown by minimum hemolytic dose [7] values of 0.0020 cc. or less. Observations in monkeys and rabbits with horse serum streptolysin S materials were compared with effects of horse serum injections.

5. Robinson, J. J.: Arch. Pediat. **61**:6 and 564, 1944; **62**:387, 1945.

6. Weld, J. T.: J. Exper. Med. **59**:83, 1934; **61**:473, 1935.

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In the preliminary work, animals were studied by means of complete gross and microscopic observations, numerous electrocardiographic tracings, and recordings of weight and temperature. Weekly blood specimens were obtained from the femoral vessels of the monkeys for studies of erythrocyte sedimentation rates, white blood cell total and differential counts, and some antistreptolysin O, hyaluronidase inhibition and Weltmann coagulation tests.

Part 2.—In the second part of the study, more extensive observations entailing fewer variables were made in rabbits. One uniform lot of 170 young albino rabbits of both sexes was obtained. Their weights at the beginning of the experiments ranged from 1,970 to 2,150 Gm. They were kept together in large pens, fed an adequate stock diet of alfalfa, rabbit chow [8] and water ad libitum, and all were uniformly handled. Numbers were tattooed in their ears. Weekly observations of rectal temperature and weight were made.

Various treatment groups consisted of at least 10 rabbits per group (table 1). Animals were assigned to treatment groups by selecting at random from shuffled cards bearing the animal numbers. Rabbits were killed by air embolism, autopsies made and histologic sections prepared. These sections were given a code number unknown to the observing pathologist, and lesions were scored on charts. Grading was from 0 to 4, extending from no abnormality to extensive change. At the conclusion of the study, reports were decoded and group data assembled from the scores.

From the femoral vessels 10 cc. blood specimens were aseptically taken for serum on days 0, 31 and 62; at the time of death, generally day 110, larger volumes of blood were obtained from

TABLE 1.—*Experimental Treatment of Groups 1 to 9*

Group	Rabbits	Agent Administered	Dose, Cc./Kg.
1	21	None	...
2	10	Sterile defibrinated blood medium	1.0
3	10	NY-5 strep. in defibrinated blood medium *	0.4
4	10	NY-5 strep. in defibrinated blood medium + penicillin *	0.2
5	10	NY-5 strep. in Difco brain-heart broth *	1.0
6	10	Viable diphtheroid cultures	1.0
7	10	Killed diphtheroid cultures	1.0
8	10	Unheated throat-washing filtrates	1.0
9	10	Heated throat-washing filtrates	1.0

* Viable streptococcus NY-5 counts were 1×10^8 on materials for group 3, 2×10^8 on those for group 4, and 4×10^7 on those for group 5. The above doses permitted uniform administration of 4×10^7 streptococci per kilogram.

the heart. Antistreptolysin O and S titrations were made on these sterile serums by methods employed in the laboratory,[9] with all specimens from an animal run in one test series with standard serums.

All rabbits in groups 1 through 9 were uniformly conditioned with intramuscular injections of Blackmore (group A, type 11), 98 and NY-5 strains of streptococci during the 110 days of the study. The streptococci were grown for 16 hours at 37 C. in Difco brain-heart broth, giving approximately 2×10^8 viable streptococci per cubic centimeter. The following schedule was observed:

1. During the first two months the streptococcal strains were administered alternately for 10-day periods in order of Blackmore, 98 and NY-5, with an initial dose of 0.5 cc. per kilogram, followed within 10 days with two injections of 1 cc. per kilogram.

2. During the last seven weeks of the study the strains were pooled and administered in weekly injections of 1 cc. per kilogram.

After completion of the first two months of conditioning treatment and concurrently with the last seven weeks of continuing treatment, the rabbits were randomly designated by groups and the agents listed in table 1 administered intravenously twice a week.

8. The chow used was that produced by the Ralston Purina Company, St. Louis.

9. Robinson, J. J.: The Technic and Significance of Antistreptolysin O and Antistreptolysin S Determinations, Research Project NM 005 051.07.01, NMRU 4, United States Department of the Navy, Great Lakes, Ill., Nov. 1950.

The defibrinated blood medium employed in groups 2, 3 and 4 was prepared from the following ingredients. It chiefly differed from the previously described streptococcal filtrate medium by having higher potassium ion concentrations and different liver extract.

Disodium phosphate, anhydrous, C. P.	23.6 Gm.
Citric acid, U. S. P.	1.1 Gm.
Dextrose, C. P.	4.4 Gm.
Magnesium sulfate U. S. P. (Epsom salt)	4.4 Gm.
Potassium nitrate, C. P.	2.2 Gm.
Potassium chloride, C. P.	15.5 Gm.
Liver extract (Lederle, 10 units/cc.)	8.0 cc.
Distilled water, to make	4,000 cc.

Ingredients were dissolved without heating and sterilized by Seitz filtration. The medium was isotonic, had a pH of 7.5 to 7.6, and was used with 10 per cent defibrinated normal rabbit blood. A portion of the medium was incubated for 10 hours at 37 C., dispensed in sterile capped vials, stored in solid carbon dioxide, gently thawed just prior to use and then maintained at 0 C. during the time it was being injected into the rabbits of group 2. Materials given groups 3, 4 and 5 were similarly kept cold.

A second portion of this medium was inoculated with a 1 per cent 16-hour broth inoculum of streptococcus NY-5. After being incubated for 10 hours at 37 C., this culture was dispensed and frozen in solid carbon dioxide. It was given to group 3. Another culture portion had 5 units of penicillin G per cubic centimeter of four-hour culture introduced; incubation continued, and after a total of 10 hours the culture was similarly stored in solid carbon dioxide. This material was given to group 4. Difco brain-heart infusion broth was identically inoculated; the culture was grown for 10 hours at 37 C. and then frozen for the material administered to group 5.

Group 6 received diphtheroid cultures of low virulence. The organism grew slowly, was very pleomorphic, formed mucoid aggregates in the 48-hour Difco brain-heart broth employed, and was not injurious to mice when 0.5 cc. of the cultures was intraperitoneally injected. Group 7 received portions of cultures given group 6 which were heated to 100 C. for 15 minutes. The purpose of group 6 was to see whether infectious granulomas might be produced at sites of carditis arising during the streptococcal conditioning.

The treatment of group 8 likewise was an attempt to observe possible superimposed inflammatory lesions at areas of streptococcal carditis, with the idea that viable filter-passing agents possibly present in throat-washing materials might be pathogenic in conditioned tissues. Materials consisted of equal parts of pH 7.2, phosphate-buffered saline and sterile Difco brain-heart infusion broth used to wash the throat and pharynx of a normal person. The washings were immediately filtered with vacuum through a 6 cm. Seitz filter at 2 C., tested for sterility with blood agar plates and semisolid thioglycollate medium, and used after 18 to 24 hours' storage at 2 C., when sterility tests for bacteria were negative. Group 9 received portions of such filtrates heated to 100 C. for 15 minutes.

Three groups of rabbits which were not conditioned with streptococci were given one intravenous injection of approximately 4×10^7 NY-5 streptococci per kilogram. These were portions of the materials injected intravenously into rabbits of groups 3, 4 and 5, prepared with three types of mediums: Group 10 (11 rabbits), defibrinated blood medium; group 11 (10 rabbits), defibrinated blood medium plus penicillin; group 12 (10 rabbits), Difco brain-heart infusion broth. Owing to the severity of the reactions of the animals, observations varied from one to 26 days, with approximately 50 per cent dying within five days and the remaining rabbits being killed at 26 days.

Two groups of 16 rabbits each served as controls. The dosage schedule employed in conditioning groups 1 through 9 was observed, and the following agents were administered: Group 13, sterile Difco brain-heart broth; group 14, killed streptococci. The killed streptococci were portions of cultures given to groups 1 through 9 which were grown in the medium given to group 13, and which were killed by heating at 100 C. for 15 minutes.

Group 15 consisted of 16 untreated rabbits selected at random from the animals to be employed in the study and killed to determine the degree and nature of any pathologic changes present prior to treatment.

Group 16 consisted of 11 rabbits killed after two months of the intramuscular streptococcus treatments given groups 1 through 9. In group 16 the purpose was to determine lesions present after two months of the streptococcal conditioning.

Group 17 consisted of 11 rabbits dying from intercurrent diseases during the first two months of the intramuscular streptococcus treatments. Nine in this group died from Pasteurella multocida bronchopneumonia.

RESULTS

Part 1.—The preliminary screening studies indicated that in rabbits subjected to sterile streptococcal culture filtrate or serum streptolysin S materials considerable carditis developed. When viable alpha or beta hemolytic streptococci were given in addition to filtrate materials, somewhat more extensive carditis occurred. Monkeys were less susceptible to this injury and exhibited relatively mild carditis. Further evidence of a species difference in reaction to streptococcal infection between monkeys and rabbits was the lack of septic arthritis in monkeys when proportional amounts of streptococci producing rabbit arthritis were given.

In nature the carditis was in part similar to human rheumatic fever, with collagenic changes coming under the description of fibrinoid degeneration, and increased number of myocytes and various degrees of inflammatory carditis. It differed from rheumatic carditis in not exhibiting the Aschoff body and in not progressing after cessation of injections. Monkeys given intranasal injections of streptococci remained well but exhibited slight rises in antistreptolysin O. No evidence of increased resistance was observed during prolonged injections of streptococcal toxic materials. Such materials possessed toxic properties causing cardiovascular injury only when kept at low temperatures, indicating the presence of heat-labile factors.

Sterile, filtered serum preparations of streptococci containing streptolysin S produced immediate, extensive carditis. Normal serum used for such preparations led to cardiovascular injury of the type observed by many investigators of serum sickness. However, normal serum had to be given intravenously and in much larger amounts than hemolytic streptolysin S materials, and required an initial immunizing period of several days, to produce this injury. Serum sickness injury was characterized by extensive arteritis resembling polyarteritis nodosa more than rheumatic fever. The serum streptolysin S preparations produced cardiac lesions somewhat lacking in the extensive cellular exudate observed when sterile, whole streptococcal culture filtrates were employed. Closer approximations to rheumatic carditis were achieved with the crude filtrate materials than with streptolysin S serum preparations. Streptococcal infection superimposed on the presumably toxic filtrate injury elicited even more cellular, proliferative cardiac lesions, which are exemplified in figure 1. These results warranted further study of streptococcal toxic and infectious injury factors and initiated the streptococcal conditioning studies of part 2.

Electrocardiograms of animals with extensive carditis were frequently normal. When abnormalities were present, they consisted primarily of delayed conduction times and occasional heart block. In the presence of considerable streptococcal infection, monkeys showed elevated erythrocyte sedimentation rates, white cell counts, antistreptolysin O titers and antistreptococcal hyaluronidase titers. Increased titers against staphylococcal hyaluronidase were absent. A general Weltmann serocoagulation shift to the left coincided with elevated erythrocyte sedimentation rates in sick monkeys.

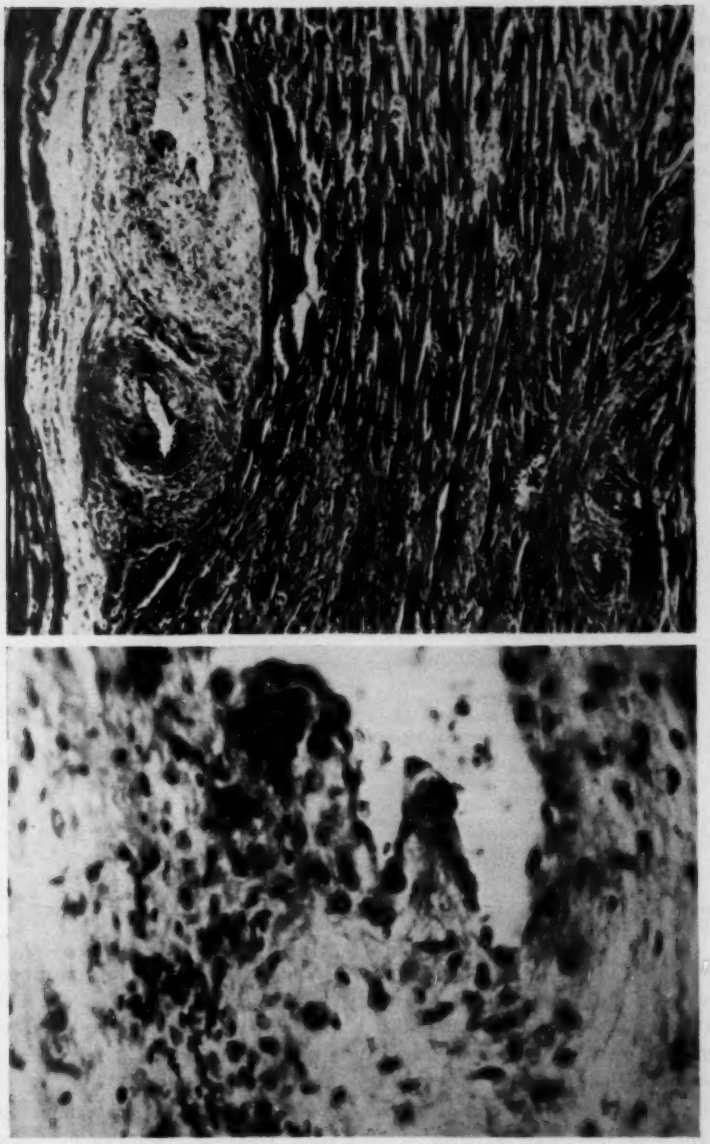

Fig. 1.—Above: Heart of a rabbit treated for 67 days with streptococcus NY-5 culture filtrates and viable streptococci. Extensive subacute carditis with areas of fibrosis, round cells, coronary arteritis and subendocardial edema. × 80.

Below: Higher magnification of an endocardial area seen in the photomicrograph above. Numerous myocytes and enlarged, multinucleated cells are associated with connective tissue edema. × 390.

Part 2.—Groups 3 and 4 exhibited the most severe carditis, as shown in tables 2 and 3. These rabbits received streptococcal conditioning and superimposed intravenous injections of 10-hour, special defibrinated blood medium cultures of the NY-5 streptococcus, with group 4 differing from group 3 in having 5 units per cubic centimeter of penicillin G added after four hours of culture growth. During the time of young culture intravenous injection, these rabbits became weak, febrile, listless, lost weight (table 4) and showed development of arthritis. Rabbits in group 1 receiving only the streptococcal conditioning treatment, those in group 2 receiving this conditioning plus intravenous injections of the sterile defibrinated

TABLE 2.—*The Extent of Histopathologic Carditis in Groups of Rabbits Treated with Streptococcal or Control Materials*

Carditis Score		Treatment			No. in Group	No. of Group
Mean	S.D.	Agent	Route	Days		
50	10	3 strains streptococci, NY-5 strep. in blood medium (toxic factors?)	IM IV	1-110 62-110	10	3
53	15	3 strains streptococci, NY-5 strep. penicillin in blood medium (toxins?)	IM IV	1-110 62-110	10	4
25	12	Single dose of NY-5 strep. in brain-heart medium (no toxic factors?)	IV	1-26	10	12
25	9	3 strains streptococci, killed diphtheroids...	IM IV	1-110 62-110	10	7
21	14	3 strains streptococci, sterile blood medium	IM IV	1-110 62-110	10	2
21	10	3 strains streptococci, NY-5 strep. in brain-heart medium (no toxins?)	IM IV	1-110 62-110	10	5
21	7	Single dose of NY-5 strep. in blood medium (toxic factors?)	IV	1-26	11	10
21	14	3 strains streptococci, viable diphtheroids...	IM IV	1-110 62-110	10	6
20	9	3 strains streptococci, throat washing filtrate	IM IV	1-110 62-110	10	8
18	12	Killed streptococci	IM	1-110	16	14
18	7	3 strains streptococci.....	IM	1-110	21	1
15	10	3 strains streptococci, heated throat filtrate	IM IV	1-110 62-110	10	9
14	8	Sterile brain-heart broth.....	IM	1-110	16	13
14	8	Single dose of NY-5 strep. in blood medium + penicillin (toxins?)	IV	1-26	10	11
12	10	3 strains streptococci, death from intercurrent disease	IM	1-62	11	17
10	7	3 strains streptococci.....	IM	1-62	16	16
2	3	None	...	1-4	16	15

Abbreviations: S.D., standard deviation about the mean; IM, intramuscular; IV, intravenous; days, duration of treatment.

blood special medium, and those in group 5 receiving the conditioning plus identical intravenous injection of young NY-5 streptococcus cultures grown in Difco brain-heart infusion broth did not manifest such severe disease as groups 3 and 4. Rabbits in groups 3 and 4 were not significantly different from each other, indicating absence of penicillin induction of a pathogenic "L" form.

Except for groups 3 and 4, other groups of rabbits were quite similar when comparisons were made in those of equal age. This was confirmed by statistical "t" tests of group carditis and adrenal hyperplasia. Thus, group 15 killed at the beginning of the study was different from other animals observed for 110 days, and rabbits kept for this period of time did not appreciably vary between themselves. Multiple strain streptococcal infection under these conditions did not produce

TABLE 3.—*Details of the Carditis, Expressed in Group Mean Histopathologic Scores × 10, Observed in Representative Groups of Rabbits Treated with Streptococcal or Control Materials*

Observation	Group No. (See Table 1)							
	3	4	2	5	14	1	13	15
Pericardium								
Round cells	19	15	4	3	4	6	1	0
Myocardium								
Muscle:								
Degeneration	27	25	13	15	12	10	9	3
Necrosis	23	16	5	6	5	3	3	0
Connective tissue:								
Polymorphonuclears	14	14	5	6	7	5	5	0
Round cells	29	29	16	18	20	17	15	8
Eosinophils	12	8	1	1	11	1	1	0
Myocytes	26	23	9	8	11	9	8	1
Degeneration	24	25	9	11	10	8	9	3
Fibrinoid degeneration	13	11	1	0	0	0	0	0
Edema	16	12	6	2	1	1	1	0
Fibrosis	20	12	4	8	6	10	3	0
Endocardium								
Polymorphonuclears	10	9	6	2	3	1	1	0
Round cells	20	21	8	8	8	7	4	1
Eosinophils	4	4	1	1	1	1	0	0
Degeneration	21	20	10	7	6	6	5	0
Fibrosis	13	11	4	7	5	9	1	0
Coronary Artery								
Intima	38	35	4	0	0	0	2	0
Media	78	73	19	19	16	15	18	1
Adventitia	96	86	31	35	30	30	21	3
Fibrinoid degeneration	12	11	0	0	0	0	0	0

TABLE 4.—*Mean Adrenal and Body Weight Changes in Groups of Rabbits Treated with Streptococcal or Control Materials*

Adrenal Histopath. Score × 10		Adrenal Wt. × 10^5 / Body Wt.	Body Wt. Gain, Gm./Day	Group No. (See Table 1)
Mean	S.D.			
34	13	25	2.4	3
33	18	25	3.9	4
28	15	21	*	12
16	11	18	10.7	6
15	14	15	12.7	13
15	10	17	8.3	2
14	16	20	6.9	5
13	12	20	11.6	16
11	11	15	*	10
11	11	14	9.5	9
11	14	14	12.6	14
11	14	15	*	11
10	11	15	*	17
10	14	15	10.6	7
9	10	16	9.0	8
8	13	11	*	15
7	16	15	10.2	1

Abbreviations: S.D., standard deviation about the mean; wt., weight; * inadequate data because of brief observation period.

extensive carditis. No effects of the diphtheroid or throat-washing filtrate injections were noted. Group 13 receiving injections of sterile broth and group 14 receiving killed streptococci may be used for comparison with rabbits receiving viable streptococci. When these groups are compared with group 1 receiving multiple strain intramuscular injections of viable streptococci, no marked differences are seen.

Table 3 summarizes details of the histopathologic carditis observed in representative groups. Fibrinoid degeneration was almost entirely limited to groups 3 and 4. Extensive pancarditis and coronary arteritis existed in these two groups, which closely resembled the carditis observed in human rheumatic fever but differed in not presenting the Aschoff body. Groups 3, and 4 and 5 exhibited considerable septic arthritis, while other rabbits conditioned with streptococci had very little arthritis. Figures 2 and 3 exemplify the carditis observed in groups 3 and 4.

Groups 10, 11 and 12, consisting of normal rabbits not previously treated with streptococci, became severely ill soon after the injection of the young streptococcus NY-5 culture materials. Even though the single dose given was identical to that repeatedly given groups 3, 4 and 5, about half of them died within five days. Those that survived exhibited extensive septic arthritis but had a rapid convalescence. Survivors were killed at 26 days. The carditis observed in these groups of nonconditioned rabbits was similar in degree to that in group 1, and much less severe than that in groups 3 and 4. No significant differences were observed among groups 10, 11 and 12.

Adrenal hyperplasia (table 4) was correlated with severity of carditis (tables 2 and 3). This hyperplasia was largely in the zona fasciculata of the adrenal cortex. The histopathologic picture was nonspecific, and the cortical hyperplasia observed was similar to that reported for various infectious diseases and stress stimuli.

Intercurrent diseases among rabbits were a minor problem. Nine rabbits died from a purulent subacute pneumonitis in which pure cultures of Pasteurella multocida were isolated (group 17). These rabbits did not show significant carditis to accompany this type of infection. A more prevalent, nonfatal disease was a syndrome of subacute encephalitis and nephritis. This was present rather uniformly in 30 of 153 rabbits used in preliminary studies conducted at Dublin, Ga., and in 113 of 201 rabbits studied in the latter part of the investigation conducted at Great Lakes, Ill. Rabbits were obtained locally. The encephalitis was characterized by foci of degeneration and subacute inflammatory foci of round cell accumulation in the central nervous system. The nephritis was similar to human pyelonephritis with focal tubular degeneration, occasional hyaline casts, interstitial round cell accumulations, and fibrosis. Glomeruli were seldom involved, and arteriolar sclerosis was absent. Sections of the bladder, the renal pelvis or the ureters were normal, and the inflammatory changes seemed to arise from hematogenous infection rather than from ascending infection of the urinary tract. Occasionally rabbits severely affected would also have increased myocardial foci of round cells and mild focal coronary arteritis. No infectious organisms were observed within the tissues or were cultivated when tissues were inoculated in blood agar or semisolid thioglycollate mediums. The etiology of this encephalitis-nephritis combination was obscure; it appeared similar to reports of Encephalitozoon cuniculi [10] infection of

10. Goodpasture, E. W.: J. Infect. Dis. **34**:428, 1924.

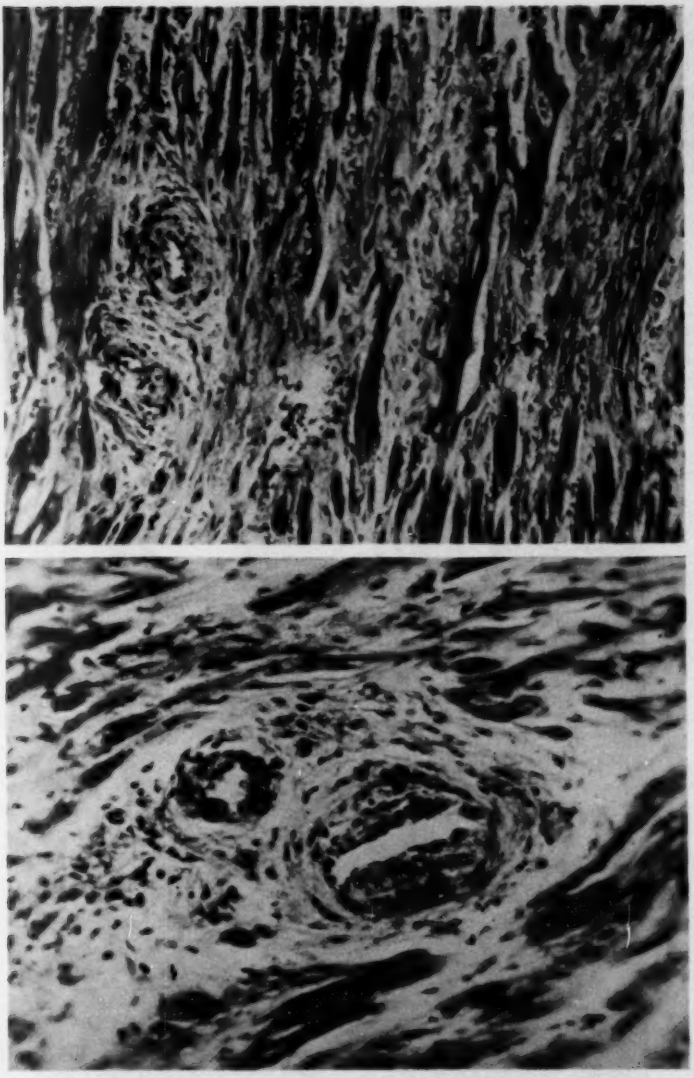

Fig. 2.—Above: Myocardium of a rabbit treated with streptococcal toxic and infectious materials (group 3). Extensive subacute carditis with myocardial and arteriolar changes. × 110.

Below: Small coronary arterioles in a rabbit treated with streptococcal toxic and infectious materials (group 3). The perivascular connective tissue edema and a multinucleated giant cell suggest similarity to human rheumatic carditis, although this is not considered a typical Aschoff body. × 270.

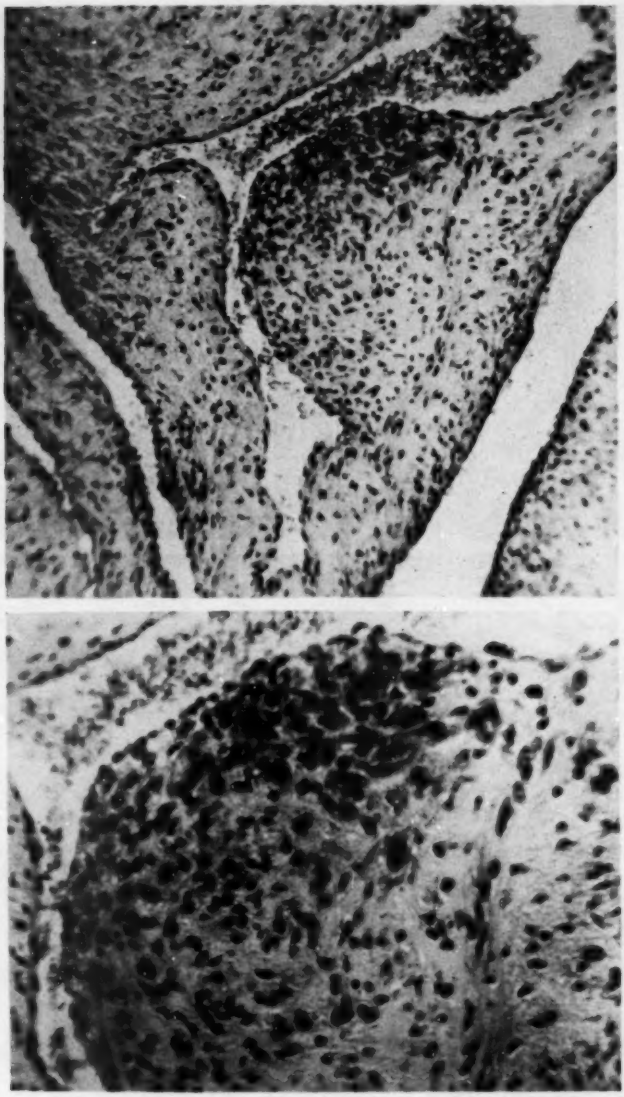

Fig. 3.—Above: Mitral valve of a rabbit treated with streptococcal toxic and infectious materials (group 4). Considerable thickening, with fibrosis and subacute inflammation. × 90.

Below: Higher magnification of a portion of the field seen in the photomicrograph above, showing the presence of large, multinucleated cells and round cells characteristic of this subacute inflammation. × 210.

rabbits, which some authors regard as toxoplasmosis.[11] Sections of brain and kidney were essential in revealing it.

Renal changes were most marked in rabbits having the encephalitis-nephritis syndrome and were uniformly distributed in the various rabbit treatment groups. Severe nephritis or vascular renal changes did not accompany the extensive carditis observed in groups 3 and 4. These two groups of rabbits manifested more changes in other organs than the rest of the rabbit treatment groups, but such changes were not uniquely associated with streptococcal injury. For example, bone marrow and lymphoid hyperplasia would reflect nonspecific reactions to injury. In comparing changes among the major organs, the disproportionately severe carditis in groups 3 and 4 was most impressive.

Table 5 summarizes the antistreptolysin O and S titers for representative groups of rabbits. Rabbits in groups 7, 8, 9 and 16 gave results similar to those in groups 1, 2 or 6, and groups 13 and 15 were like group 14. No significant differences in antistreptolysin S titers occurred between groups, but there was an apparent decrease from day 0 to 110 in all groups. The antistreptolysin O titers in rabbits

TABLE 5.—*Mean Antistreptolysin O and S Titers in Units per Cubic Centimeter of Serum for Representative Groups of Rabbits Treated with Streptococcal or Control Materials*

	Antistreptolysin O				Antistreptolysin S*			
	Day of Specimens				Day of Specimens			
Group (See Table 1)	0	31	62	110	0	31	62	110
1	4	31	73	34	38	21	20	20
2	5	48	96	45	29	19	21	19
3	3	30	57	82	32	20	21	19
4	2	17	55	85	34	19	20	16
5	3	14	107	106	31	21	22	14
6	2	18	61	39	33	23	23	18
14	5	3	7	10	32	25	21	18

* One unit of antistreptolysin S in these determinations equals 0.4 unit of Todd's[7] antistreptolysin S.

given intramuscular injections of viable streptococci rose to a maximum on day 62 and then declined; rabbits in groups 3, 4 and 5 receiving intravenous injections of the NY-5 streptococcus from days 62 to 110 showed increasing titers.

COMMENT

The multiple strain group A streptococcal infection approach employed in this study differed from the method of Murphy and Swift.[4] Those workers used small intracutaneous inoculations, which resulted in carditis in a minor number of rabbits. It was considered desirable to employ larger amounts of streptococci to afford more prevalent and extensive carditis. While the 98 strain was untypable both in this laboratory and by Rammelkamp,[12] who originally isolated it, and hence might have been of the same type as the Blackmore or NY-5 strains, it differed from the Blackmore in producing streptolysin O and from the NY-5 in forming scant erythrogenic (Dick) toxin and may be considered different from these other strains. The pro-

11. Hagan, W. A.: The Infectious Diseases of Domestic Animals with Special Reference to Etiology, Diagnosis, and Biologic Therapy, Ithaca, N. Y., Comstock Publishing Co., Inc., 1943.

12. Rammelkamp, C. H.: Personal communication to the author.

longed and fairly intensive intramuscular administration of these three group A strains did not result in severe carditis (tables 2 and 3).

Results indicated that a heat-labile, nonantigenic factor associated with young NY-5 strain streptococcus cultures grown in a special defibrinated blood medium could produce carditis in rabbits. In the preliminary studies, streptococcal filtrate exhibited heat instability similar to that of streptolysin S. Such materials did not possess appreciable hemolytic properties, as evidenced by minimum hemolytic dose titers of over 1.0 cc. for filtrates in the preliminary study and the special materials given groups 2, 3 and 4. The special material given group 5 consisting of a 10 hour NY-5 streptococcus growth in Difco brain-heart broth had a minimum hemolytic dose value of 0.20 cc. but was lacking in this toxic factor as judged by the low degree of carditis. Hence, free streptolysin S could not explain the toxic action, since it would have given a greater hemolytic potency. The special defibrinated blood medium alone was innocuous as seen (tables 2 and 3) by the absence of marked carditis in group 2 receiving this material, which likewise indicated an absence of foreign protein injury. Also, rabbits were ill promptly after the injection of these toxic materials and maintained the same response levels during the study, an observation again indicating an agent differing from antigenic foreign substances or from serum injury mechanisms requiring several days for development.

These studies have not disclosed the nature of the toxic factor, or factors, associated with young streptococcal cultures grown in special defibrinated blood mediums but absent when Difco brain-heart broth is employed. The increased carditis observed in groups 3 and 4 apparently resulted from such toxic factors, which one might guess could be similar to the toxic albumin-bacterioplasma conjugates reported by Schultz and Rose [13] or the cardiac injuring factor which Schultz and Fite [14] observed in the serums of rheumatic fever patients. Further studies are planned to develop initially methods for toxin detection that would be superior to the cumbersome assays of carditis in rabbits and subsequently other methods by which the obscure toxic factor might be fractionated and the fractions identified.

Normal rabbits not conditioned by streptococcal infection, comprising groups 10, 11 and 12, which received a single injection of the special materials given groups 3, 4 and 5 reacted differently from rabbits subjected to previous streptococcal infection. Essentially the same degree of carditis was observed throughout groups 10, 11 and 12. This carditis was not very extensive and was characteristic of the usual carditis observed in acute streptococcal septicemia. This indicated a difference in response between immune and nonimmune animals and suggested that the immune groups (3, 4 and 5) were protected from fatal streptococcal septicemia and handled certain streptococcal products differently. Possibly there is in immune animals increased tissue fixation of poorly antigenic streptococcal products or conjugates, with resultant tissue injury analogous to the positive tuberculin reaction in tuberculous animals. This problem could be investigated by means of radioisotope technics.

The histologic character of the carditis in groups 3 and 4 (table 2) was distinct from that of the carditis in other groups by the presence of fibrinoid degeneration

13. Schultz, M. P., and Rose, E. J.: Pub. Health Rep. **62**:1009, 1947.

14. Schultz, M. P., and Fite, G. L.: Am. J. Path. **26**:706, 1950.

and numerous inflammatory changes resembling human rheumatic fever carditis. The Aschoff body was absent, however, and no claims for the experimental duplication of rheumatic fever can be made. The data indicated that in rabbits subjected to streptococcal infection for a period and then treated with potentially toxic streptococcal factors a severe carditis developed which was more like rheumatic fever than the carditis associated with multiple strain group A streptococcal infection. The diphtheroid organism, Pasteurella multocida, possible filter-passing agents in throat washings, and the unknown agent causing rabbit encephalitis-nephritis did not elicit significant carditis. These observations agreed with previous reports [15] concerning the somewhat selective cardiac injury associated with streptococci in rabbits.

Since groups 3 and 4 gave essentially identical results, no evidence could be found for a penicillin-induced formation of "L" or pleuropneumonia-like variants of the NY-5 streptococcus which possessed pathogenic properties under these circumstances. The reason for an inability to kill established NY-5 cultures in the special defibrinated blood medium with penicillin in amounts up to 5,000 units per cubic centimeter was not determined. It is possible that a penicillinase formed in such cultures was responsible for this observation. The NY-5 strain had the usual penicillin sensitivity of group A streptococci, since growth was entirely prevented when the antibiotic had been introduced into the medium to the amount of 0.10 unit per cubic centimeter prior to inoculation.

Antistreptolysin S titers were determined during the fifth month after the onset of the rabbit group study, and hence initial serum specimens were stored about four months longer than the last. Serums were kept aseptically in rubber-capped vials at 4 C. The finding that the initial specimens had higher antistreptolysin S titers than the later ones in all groups (table 5) can most likely be attributed to storage factors. Humphrey [16] and Stollerman and co-workers [17] have shown that serum inhibitors of streptolysin S were unstable and were increased by certain mild denaturing procedures. These data indicated that streptolysin S associated with streptococcal infection was nonantigenic, in contrast to an earlier report of Todd [7] that streptolysin S was antigenic when associated with viable streptococci. Previous tests showed that the Blackmore, 98 and NY-5 strains all formed considerable streptolysin S in vitro. Antistreptolysin O and S titers were not correlated with severity of carditis, and antistreptolysin O titers merely reflected the degree of streptolysin O antigenic exposure.

SUMMARY

Severe carditis was observed in rabbits into which group A streptococci and their presumably toxic products had been injected. This carditis seemed to depend on the immune state of the animals and the presence of an unknown heat-labile, nonantigenic, nonhemolytic toxic factor, or factors. It was observed in detail in rabbits receiving intramuscular injections of streptococci and after two months additional intravenous injections of a 10-hour culture of NY-5 streptococci grown in a special defibrinated blood medium.

15. Wilson and Miles.[8] Robinson.[5]

16. Humphrey, J. H.: Brit. J. Exper. Path. **30**:345, 1949.

17. Stollerman, G. H., and Bernheimer, A. W.: J. Clin. Invest. **29**:1147, 1950. Stollerman, G. H.; Bernheimer, A. W., and MacLeod, C. M.: Ibid. **29**:1636, 1950.

Sterile culture filtrates or serum extracts of certain group A streptococci produced an immediate, severe carditis in rabbits.

Macacus mulatta (rhesus) monkeys were more resistant than rabbits to group A streptococci and their products.

Extensive and prolonged intramuscular injections of three group A strains of streptococci did not produce severe carditis in rabbits.

Adrenal hyperplasia was associated with the severity of carditis in rabbits subjected to streptococcal injury.

Rabbits infected with group A streptococci known to produce streptolysin S did not present increased serum inhibitors for streptolysin S. Increased antistreptolysin O was correlated with the antigenic exposure to streptolysin O but not with the severity of carditis.

Attempts to produce rheumatic carditis in monkeys and rabbits were unsuccessful, since typical Aschoff bodies were not observed. However, certain approximations of this goal were attained which suggested that group A streptococci and their products might be involved in the genesis of rheumatic fever.

The staff of Naval Medical Research Unit No. 4, under the direction of Comdr. J. R. Seal (MC) U. S. N., have given aid in many phases of these studies. Lt. C. E. Curtis has contributed statistical assistance. Dr. Howard T. Karsner helpfully reviewed the manuscript.

EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS

III. The Reaction of the White Blood Cells

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THE REACTION of blood cells in experimental cholesterol arteriosclerosis has already been studied, but such results as have been recorded have not been consistent. While some observers have maintained that no hematologic changes occur, others [1] have mentioned anemia. Okey and Greaves [1b] reported leukocytosis but did not elaborate this finding. With the exception of Okey and Greaves, who examined the blood of guinea pigs, these investigators used rabbits for their studies. A previous report from this department [2] indicated that blood cell counts and hemoglobin values remained normal in cholesterol-fed rabbits, but, as was mentioned at that time, the number of animals examined was insufficient. In view of the marked tissue changes observed in all rabbits, even after a relatively short period of cholesterol administration, some doubts arose as to the validity of the negative statements, and a more systematic investigation was considered advisable.

Similar experiments were also undertaken on rats, which are not susceptible to cholesterol arteriosclerosis.

MATERIAL AND METHODS

In making the total and differential leukocyte counts, the usual precautions were taken; the determinations were made at the same time of the day, by the same person (M. E. Martin), and the same chamber and pipettes were used.

In the experiments involving rabbits, blood was taken from the marginal ear vein. Counts made before the experiments started were usually under 10,000 cells per cubic millimeter, and in no case did they exceed 12,000 cells per cubic millimeter. Systematic examinations of the white blood cells were made in 10 rabbits for periods varying from 50 to 280 days, excluding the control periods. In two more rabbits, the counts were begun after the animals had been receiving cholesterol for 10 months. Two methods of feeding were employed. In one the rabbits received a commercial brand of calf meal pellets, oats, some greens, and gelatin capsules

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1. (*a*) Ignatowsky, A.: Über die Wirkung des tierischen Eiweisses auf die Aorta und die parenchymatösen Organe des Kaninchen, Arch. path. Anat. **198**:248, 1909. (*b*) Okey, R., and Greaves, V. D.: Anemia Caused by Feeding Cholesterol to Guinea Pigs, J. Biol. Chem. **129**:111, 1939. (*c*) Dubach, R., and Hill, R. M.: The Effect of a Sustained Hypercholesterolemia on the Lipids and Proteins in the Plasma of the Rabbit, ibid. **165**:521, 1946.

2. Altschul, R.: Heterotopic Blood Formation in Experimental Cholesterol Arteriosclerosis, Arch. Path. **44**:282 (Sept.) 1947.

containing 0.45 Gm. of pure cholesterol daily; in the other they were given a diet of milk, yolk powder and yolk cake ad libitum. In some animals the milk-yolk diet was supplemented at certain periods with doses of pure cholesterol. Details of this diet have been given elsewhere.[3] Later in the experiments, when somewhat rhythmic changes in the white blood cell counts became apparent, it was deemed advisable to determine the serum cholesterol levels. This was done (Miss M. E. Mahon) by means of the Sperry-Schoenheimer method as modified by C. S. McArthur. In addition, immature forms of red blood cells appearing in the blood of some rabbits during the later course of the experiments prompted determinations of hemoglobin values.

In the experiments involving rats, blood was obtained by cutting the tip of the tail, with the rat under light ether anesthesia. In an attempt to determine influences of the basic diet, preliminary counts were made on all rats while they were fed a high carbohydrate diet. Some animals were then placed on a high protein diet and again the blood counts were determined. Thereafter eight of these animals received the protein diet supplemented with 0.1 Gm. of pure cholesterol per day, or a milk and yolk diet. Further control observations were made by feeding the animals the control and the experimental diets alternately.

RESULTS

In all rabbits, the administration of pure cholesterol or the milk-yolk diet was quickly followed by an increase of leukocytes, which continued uniformly or irregularly for a few days (charts 1 to 4). This increase was due mainly to lymphocytosis, although an initial, less marked, neutrophilia was also present in five rabbits. The further course showed in all animals a general increase in the number of white blood cells which, however, was always interrupted by lesser or major decreases. The monocytes and the eosinophils and basophils appeared to show a proportionate increase, but owing to their comparatively small numbers, the margin of error was too great to allow a more definite statement. In two cases the number of basophils increased markedly for short periods (up to 3,000 cells per cubic millimeter).

The highest total leukocyte value reached was 33,000 cells per cubic millimeter. The highest value for lymphocytes was 15,900 cells per cubic millimeter, and the highest for neutrophils 22,000 per cubic millimeter.

The reactions of the white blood cells were quite irregular on first sight, but by comparing the results of individual cases, a general, though variable, pattern of increase could be discerned. In some cases the increase was fairly regular; in other cases the over-all increase consisted of a number of smaller increases. Both large and small increases in the cell count were followed by decreases, for which there was no apparent reason. It seemed possible that the irregularities might be related to variations in the blood cholesterol. However, the reports on this subject[4] are quite unanimous that cholesterol feeding causes a fairly regular increase of blood cholesterol in rabbits until, after three to five months, a peak is reached. Thereafter the level decreases slowly in spite of continuous administration of cholesterol to reach the double or triple of the initial value. This observation was confirmed by our experiments. However, only a moderate number of serum

3. Altschul, R.: Experimental Arteriosclerosis in the Nervous System, J. Neuropath. & Exper. Neurol. **5**:333, 1946; Selected Studies on Arteriosclerosis, Springfield, Ill., Charles C Thomas, Publisher, 1950.

4. Thölldte, M.: Hypercholesterinämie, Blutdruck und Gefässveränderungen im Tierversuch, Beitr. path. Anat. u. allg. Path. **77**:61, 1927. Weinhouse, S. F., and Hirsch, E. F.: Atherosclerosis: Lipids of Serum and Tissues in Experimental Atherosclerosis of the Rabbit, Arch. Path. **30**:856 (Oct.) 1940.

cholesterol determinations were made lest excessive blood-taking might disturb the balance of blood formation or damage the ear veins with resulting inflammatory reaction. In spite of this we were able to establish that the aforementioned drops in the number of white blood cells were not accompanied by a lowering of the cholesterol level in the serum.

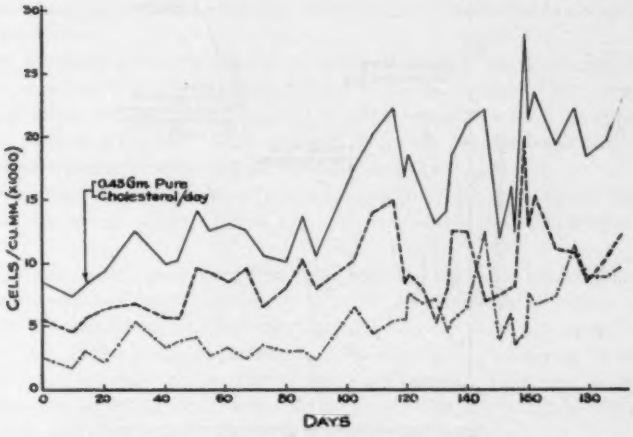

Chart 1.—Leukocyte counts on an adult rabbit fed pure cholesterol. In all charts the continuous line indicates total white blood cells per cubic millimeter; the dash line, lymphocytes, and the dash and dot line, neutrophils.

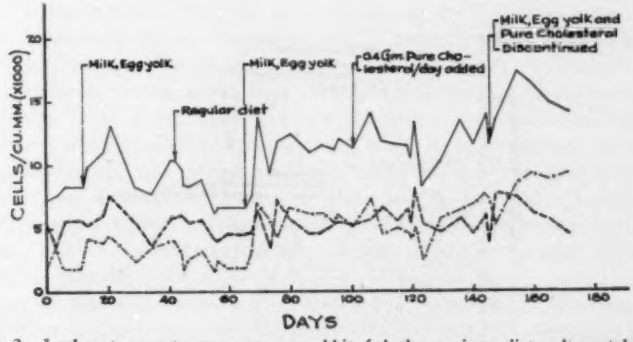

Chart 2.—Leukocyte counts on a young rabbit fed the various diets alternately; age at start 40 days.

In our control determinations the differential counts showed a predominance of lymphocytes over neutrophils. Previously, Kellum and Forkner [5] had found a similar proportion. Kracke,[6] however, gave the ratio of percentages as 41.8: 43.4;

5. Kellum, W. E., and Forkner, C. E.: The Normal Blood Picture in the Rabbit by the Vital Staining Method, Anat. Rec. **25**:109, 1923.

6. Kracke, R. R.: Diseases of the Blood and Atlas of Hematology with Clinical and Hematologic Description of the Blood Diseases Including a Section on Technic and Terminology, ed. 2, Philadelphia, J. B. Lippincott Company, 1941.

Pearce and Casey [7] also found a higher percentage of neutrophils than of lymphocytes.

The predominance of lymphocytes was maintained in the initial increase after the cholesterol or the milk-yolk diet was begun, and in most cases became even

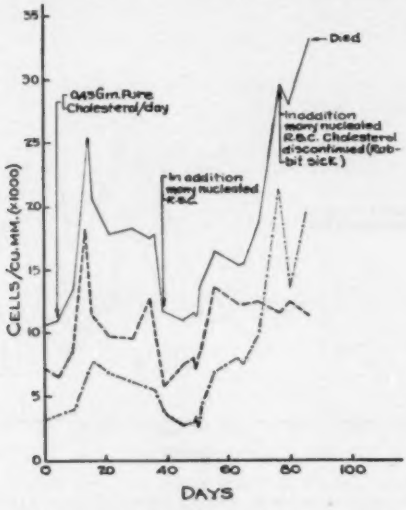

Chart 3.—Leukocyte counts on an adult rabbit in which nucleated red blood cells, indicating anemia, appeared during feeding of pure cholesterol.

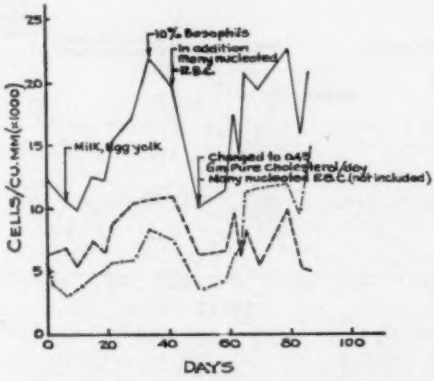

Chart 4.—Leukocyte counts on an adult rabbit in which nucleated red blood cells appeared during feeding of milk-yolk diet.

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7. Pearce, L., and Casey, A. E.: Studies in the Blood Cytology of the Rabbit: I. Consecutive Neutrophile, Basophile, and Eosinophile Observations on Groups of Normal Rabbits, J. Exper. Med. **52**:145, 1930; II. Consecutive Lymphocyte and Monocyte Observations on Groups of Normal Rabbits, ibid. **52**:167, 1930.

more marked. However, after several weeks or, more often, after several months, the number of neutrophils began to increase without marked decrease in the number of lymphocytes. In the later stages of experiments it was the neutrophilic element which contributed most to the leukocytosis.

The leukocyte counts of the animals fed the milk-yolk diet did not show as notable an increase as those of animals fed pure cholesterol. However, it was difficult to induce the animals to take the milk-yolk diet in sufficient amounts for long enough periods.

On interruption of the cholesterol or milk-yolk feeding the leukocyte numbers decreased, usually within a short time, but not to normal values. Only after a long discontinuation of the diet did the number of white blood cells show an approximation of normal, the decrease occurring not uniformly but by leaps and bounds, similar to the manner in which the increase had occurred.

In six animals the occurrence of nucleated red blood cells was noted, indicating an anemic condition (charts 3 and 4). In these cases the hemoglobin values were low.

In eight rats which were given the milk-yolk diet or cholesterol-supplemented diets definite changes in leukocyte counts were not obtained. There was some increase in the number of lymphocytes; it was more even, but less immediate and less steep, than in the rabbit experiments. The influence of milk-yolk feeding was somewhat more impressive than that of pure cholesterol. However, since rather large fluctuations appear to occur normally in the rat, interpretation of slight changes is hazardous, and these results should not be taken as final.

COMMENT

Experimental cholesterol arteriosclerosis is accompanied by an increase in the number of leukocytes in rabbits. In the first stage of the experiments the increase is mainly relative and absolute lymphocytosis; in the later stages it may change to relative lymphopenia. The neutrophilia is not surprising in view of the severe damage of tissue occurring in the later stages of experimental cholesterol arteriosclerosis, such as foci of necrosis of the liver and adrenals, atheromatous decay, etc.

The lymphocytosis, particularly its initial stage, is more difficult to explain. Various authors, especially Bergel,[8] have advanced the view that lymphocytes are involved in lipoid metabolism. This theory has been generally abandoned. Since cholesterolemia is combined with lipemia, the latter might be responsible for the lymphocytosis. But for this mere parallelism, we have at present no other evidence to show a causal relation.

Another concept which should be considered is the so-called "digestion leukocytosis." This, too, is held in disfavor today. In any case, the degrees of lipemia, cholesterolemia and leukocytosis are too high to fit this concept.

In view of the fact that in experimental cholesterol arteriosclerosis the adrenal cortex undergoes grave alterations, the reaction of the white blood cells should be considered in the light of Selye's[9] stress theory. While the neutrophilia would

8. Bergel, S.: Weiteres zur lipoidspaltenden Funktion der Lymphocyten, Beitr. path. Anat. u. allg. Path. **73**:404, 1924-1925.

9. Selye, H.: The Physiology and Pathology of Exposure to Stress, Montreal, Acta, Inc., 1950.

fit into this concept, the lymphocytosis, present almost from the beginning of the experiments, is contrary to the reaction of lymphocytes observed in the alarm reaction. It is true that lymphocytosis, as well as neutrophilia, could be explained as corresponding to conditions following adrenalectomy, but their early appearance are not in timely accord with the morphologic changes of the adrenal cortex.

Examination of the graphs in charts 1 to 4 will readily explain the previous negative statements as to the white and red blood cell picture; unless systematic determinations are made, a random sampling may give a false picture, since it may be taken at times when the count is low or at most only slightly raised and therefore may be regarded as normal or "still normal."

SUMMARY

Administration of cholesterol or feeding of a milk-yolk diet elicits in rabbits a leukocytosis which consists of an absolute and relative increase in lymphocytes and an absolute, but lesser, increase in neutrophils. The general increase occurs somewhat irregularly and is interrupted by frequent decreases. Thereafter, the reaction of the white blood cells shows a somewhat vague rhythmic oscillation, which can often be influenced by an interruption and a resumption of the cholesterol feeding. In most of the cases a relative and absolute neutrophilia is observed in the later stages of the experiments. While the latter is readily explained by tissue damage, the lymphocytosis has found no final explanation.

There is no strict parallelism between the number of leukocytes and the cholesterol level in the serum. Anemic conditions were found in about half of the cases.

The results of similar experiments on rats were less impressive and are to be accepted with reserve.

BOTULISM, A COMPLICATION OF CLOSTRIDIUM BOTULINUM WOUND INFECTION

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AND
ALBERT P. McKEE, M.D.
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BOTULISM as a result of the ingestion of the toxin of Clostridium botulinum has been recognized since Van Ermengen established its epidemiology.[1] Although a few cases in which Cl. botulinum was isolated from traumatic wounds have been reported,[2] in none were there symptoms of botulism. The following case is believed to be an instance in which botulism followed Cl. botulinum infection of a wound.

REPORT OF A CASE

History.—On March 30, 1948, a 13 year old white boy was accidentally shot in the left thigh with a shotgun loaded with "bird shot." A physician cleansed the wound, placed several deep sutures, separated the superficial layers with gauze and administered 1,500 units of tetanus antitoxin subcutaneously. The boy appeared to be progressing satisfactorily until April 4. In the evening of that day his speech was slurred, as if he talked with a "thick tongue," and he was unable to cough. During the ensuing two days he had progressive difficulty in swallowing, increasing weakness of his neck and complained of a sore throat. Mentally he became increasingly clouded; he was somnolent, disoriented and finally semistuporous. He was admitted to the University Hospitals on April 6. The boy had been perfectly well prior to the injury. He had had no convulsion, febrile illnesses, nausea or vomiting. The past history was noncontributory except for rickets at the age of 2. The only known immunization that he had received was against typhoid.

Physical Examination.—The patient appeared to be a critically ill, asthenic, poorly nourished, preadolescent white boy. His rectal temperature was 99.2 F.; pulse rate, 96 per minute; blood pressure, 110/44 mm. Hg. He was extremely drowsy but could be aroused by noxious stimuli. There was considerable mucus in the pharynx and trachea, requiring frequent aspiration. The chest, however, was clear to percussion and auscultation. The respiration rate was 28 per minute; the chest movements were shallow but fairly regular. He could move all extremities, although weakly, and the left lower extremity appeared to be somewhat weaker than the right. Examination of the cranial nerves revealed bilateral paresis of the seventh, ninth and twelfth nerves with probable paresis of the motor component of the fifth nerve. Sensory examination was impossible because of the mental depression and lack of cooperation.

From the Departments of Surgery and Bacteriology, College of Medicine, State University of Iowa.

1. Zinsser's Textbook of Bacteriology, ed. 9, New York, Appleton-Century-Crofts Company, Inc., 1948.

2. (*a*) Pulaski, E. J.; Meleney, F. L., and Spaeth, W. L. C.: Bacterial Flora of Acute Traumatic Wounds, Surg., Gynec. & Obst. **72**:982, 1941. (*b*) Hall, I. S.: The Occurrence of Bacillus Botulinus, Types A and B in Accidental Wounds, J. Bact. **50**:213, 1945.

The neck muscles were extremely weak, and the head rolled freely in all directions. The deep and superficial reflexes were equal bilaterally but diminished in magnitude. There was an intermittent positive great toe response on the right and an occasional questionable positive response on the left side.

A dirty, malodorous wound, measuring 2 cm. in diameter, involved the anterolateral aspect of the left midthigh. The periosteum was absent from the visible underlying femur. Only a narrow area of inflammation and induration was present in the immediate vicinity of the wound. There was no crepitation in adjacent soft tissues. In the left groin a few small lymph nodes were palpable.

The blood revealed a hemoglobin content of 11 Gm. per 100 ml. and 4,940,000 erythrocytes and 6,150 leukocytes per cubic millimeter. The differential count was 68 segmented and two band granulocytes and 30 lymphocytes. A roentgenogram of the left thigh demonstrated numerous "bird shot" in the soft tissues. A few "bubbles" of gas were present. There was no visible fracture. A roentgenogram of the chest was interpreted as normal. Lumbar puncture performed with the patient in the lateral decubitus position gave normal manometric readings. The spinal fluid revealed no cells, and the Pandy test was negative. The spinal fluid protein, sugar and chloride were 17 mg., 55 mg. and 688 mg. per 100 ml., respectively. A smear of the fluid failed to demonstrate organisms. The urine was examined for lead and arsenic, and none was found. The results of the routine urinalysis were within normal limits.

Course.—Débridement of the wound was done. The muscles of the thigh were red in color and of normal consistency. Many small lead pellets were palpated in the soft tissues of the thigh. A small piece of matted hair, probably part of the wadding of the shotgun cartridge, was removed. Adequate drainage was established and the wound treated with activated zinc peroxide paste in an attempt to establish an aerobic milieu.

The patient was placed in moderate Trendelenburg position and his airway kept clear of secretions by aspiration. Penicillin, 200,000 units, was given every three hours intramuscularly. Twenty thousand units of tetanus antitoxin and 20,000 units of diphtheria antitoxin were administered. Oxygen was administered through a nasal catheter. The wound was redressed with a paste prepared with medicinal zinc peroxide U.S.P.[3] twice daily. Dextrose, isotonic sodium chloride solution and protein hydrolysate were given intravenously for nutrition and hydration. One 500 cc. blood transfusion was administered.

The patient failed to improve on this regimen, and his temperature became subnormal. In the evening of April 7 his condition became more critical. The rectal temperature decreased to 95.8 F.; the pulse was rapid and weak and the skin cold and moist. Cyanosis was noted, and at this time the respirations became progressively more shallow. Death occurred April 8, nine days after the injury and four days after the onset of symptoms.

Necropsy.—The body was that of a small, asthenic, undernourished white boy of 13 years. A 10 cm. gaping wound was present along the lateral aspect of the left midthigh. The wound margins were covered with a thin layer of necrotic tissue and fibrin. A few "bird shot" were palpable in the depths of the wound. The femur at the depth of the wound was devoid of periosteum and was fractured obliquely, with no displacement of the fragments. Histologically the wound margins demonstrated acute inflammation involving the subcutaneous and muscular tissues. There was no evidence of cellular necrosis, and the degree of inflammation was less than that which is usually associated with gas gangrene.[3a] Except for a moderate degree of lobular pneumonia bilaterally, the thoracic and abdominal viscera were not remarkable.

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3. Meleney, F. L., and Harvey, H. D.: The Combined Use of Zinc Peroxide and Sulfanilamide in Treatment of Chronic, Undermining, Burrowing Ulcers Due to the Micro-Aerophilic Hemolytic Streptococcus, Ann. Surg. **110**:1067 (Dec.) 1939.

3a. Govan, A. D. T.: An Account of the Pathology of Some Cases of Clostridium Welchii Infection, J. Path. & Bact. **58**:423, 1946.

Examination of the brain and spinal cord revealed the former to be edematous and congested, weighing 2,200 Gm. A pressure cone was observed about the medulla, such that the medulla and the tonsils of the cerebellum had herniated into the foramen magnum. Multiple sections of the brain and spinal cord revealed severe edema and mild neuronophagia. There was no evidence of fat embolism.

Bacteriological Examination.—Spinal fluid and blood obtained before death failed to yield growths of organisms, and throat swab cultures showed flora normal for the throat.

Postmortem specimens included heart blood and tissue from the wound. Pseudomonas aeruginosa was cultured from the heart blood. The tissue specimen was used to inoculate cooked meat medium and iron-milk. Hemolytic Staphylococcus aureus, alpha hemolytic streptococci and nonhemolytic streptococci were cultured from the tissue specimen. Clostridium welchii and Clostridium botulinum type A were identified by specific antitoxin neutralization tests. Mice and guinea pigs were used as the test animals.

Since wadding from the shotgun shell was found in the wound from which the Cl. botulinum was isolated, an attempt was made to isolate the organism from a second shell presumably from the same carton. This attempt was unsuccessful; however, Clostridium tetani was isolated from the wadding of this second shell.

Pathology of Botulism.—There are no characteristic pathologic changes in a patient who has died of botulism. This disease has been studied clinically and experimentally by a number of independent observers,[4] the most significant findings being thrombosis, congestion and hemorrhage involving the brain and meninges. There was no evidence that these changes were specific for botulism.

Physiological investigations [5] have disclosed that the symptoms of botulism may be explained by an incomplete curare-like paralysis of motor nerves to voluntary muscles, including the diaphragm, and by more or less complete paralysis of parasympathetic nerve endings. This paralysis may be brought about by (1) prevention of the action of acetylcholine on striated muscle or (2) inhibition of the synthesis of acetylcholine.[6] The recent work of Burgen and associates [7] invokes interaction of toxin and fine unmyelinated nerve fibers entering the motor end-plate region, causing a block in the transmission of nerve impulses.

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4. Van Ermengen, cited by Zinsser.[1] Wilbur, R. L., and Ophüls, W.: Botulism: A Report of Food-Poisoning Apparently Due to Eating of Canned String-Beans, with Pathological Report of a Fatal Case, Arch. Int. Med. **14**:589 (Oct.) 1914. Dickson, E. C.: Botulism, an Experimental Study: A Preliminary Report, J. A. M. A. **65**:492 (Aug. 7) 1915. Cowdry, E. V., and Nicholson, F. M.: An Histologic Study of the Central Nervous System in Experimental Botulism Poisoning, J. Exper. Med. **39**:824, 1924. Shay, D. C.: An Histologic Study of the Effects of Experimental Botulinus Poisoning on the Livers of Guinea Pigs, J. Clin. Invest. **25**: 687, 1946.

5. Edmunds, C. W., and Long, P. H.: Contribution to the Pathologic Physiology of Botulism, J. A. M. A. **81**:542 (Aug. 18) 1923. Dickson, E. C., and Shevky, R. J.: Studies on the Manner in Which the Toxin of Clostridium Botulinum Acts upon the Human Body, J. Exper. Med. **37**:711, 1923. Edmunds, C. W., and Keiper, G. F., Jr.: Further Studies on the Action of Botulinus Toxin, J. A. M. A. **83**:495 (Aug. 16) 1924.

6. Torda, C., and Wolff, H. G.: On the Mechanism of Paralysis Resulting from Toxin of Clostridium Botulinum, J. Pharmacol. & Exper. Therap. **89**:320, 1947.

7. Burgen, A. S. V.; Dickens, F., and Zatman, L. J.: The Action of Botulinum Toxin on the Neuromuscular Junction, J. Physiol. **109**:10, 1949.

CLOSTRIDIUM BOTULINUM AND WOUND INFECTIONS

The natural habitat of Cl. botulinum is the soil. Cl. botulinum has been found to be a common soil anaerobe of the western states of the cordilleran system; it is less frequently encountered in the Atlantic seaboard states and is relatively rare in the Middle Western states, the Great Plains and the Mississippi Valley.[8] The organism has been found more prevalent in virgin soils than in dirt, soil or manure collected from animal corrals, pig pens, etc. The organism is not unusual in other parts of the world.[9]

From this widespread distribution it is seen that Cl. botulinum is a potential contaminant of practically every traumatic wound. That the organisms of the soil, primarily the Clostridium group, are a common finding in the bacterial flora of fresh traumatic wounds has long been recognized.[10] Despite the prevalence of the organisms in the soil and the high incidence of cases in which other clostridia have been isolated from wounds, only four cases have been reported in which Cl. botulinum was isolated from traumatic wounds. The explanation of this fact is not apparent, for in general, as far as the Clostridium group of organisms is concerned, the development of an infection in a wound so contaminated is dependent on conditions for anaerobic growth. Thus, Starin and Dack [11] found that detoxified spores of Cl. botulinum types A and B were capable of multiplying in the bodies of animals and of producing a potent toxin in sufficient amounts to induce botulism. Furthermore, Coleman,[12] in 1929, noted that by utilizing formaldehyde solution U.S.P. as a tissue debilitant one could produce botulism in guinea pigs by injecting as few as 25 viable heated spores of Cl. botulinum. He also called attention to the fact that in the guinea pig the growth requirements of Cl. botulinum were practically identical with those of Cl. tetani.

Several factors may be contributing to the failure to identify this organism as a wound contaminant. The phenomenon of dormancy or delayed germination of spores as noted experimentally may be important.[13] The difficulty of isolating

8. Meyer, K. F., and Dubowsky, B. J.: The Distribution of the Spores of B. Botulinus in the United States, J. Infect. Dis. **31**:558, 1922.

9. Dubowsky, B. J., and Meyer, K. F.: The Distribution of the Spores of B. Botulinus in the Territory of Alaska and the Dominion of Canada, J. Infect. Dis. **31**:595, 1922. Meyer, K. F., and Dubowsky, B. J.: The Occurrence of the Spores of B. Botulinus in Belgium, Denmark, England, The Netherlands and Switzerland, ibid. p. 600. Schoenholz, P., and Meyer, K. F.: The Occurrence of the Spores of B. Botulinus in the Hawaiian Islands and China, ibid. p. 610. Jones, M. T., and Tanner, F. W.: Incidence and Distribution of Clostridium Botulinus in Soils of Illinois, Food Research **10**:238, 1945. Tanner, F. W., and Dack, G. M.: Clostridium Botulinum: Presence in the Human Alimentary Tract, J. A. M. A. **79**:132 (July 8) 1922.

10. (*a*) Rustigian, R., and Cipriani, A.: The Bacteriology of Open Wounds, J. A. M. A. **133**:244 (Jan. 25) 1947. (*b*) Smith, L. De S., and George, R. L.: The Anaerobic Flora of Clostridium Myositis, J. Bact. **51**:271, 1946. (*c*) MacLennon, J. D.: Anaerobic Infections of War Wounds in the Middle East, Lancet **2**:63, 1943. (*d*) Altmeier, W. A.: The Bacteriology of War Wounds, Surg., Gynec. & Obst. **75**:518, 1942.

11. Starin, W. A., and Dack, G. M.: Pathogenicity of Clostridium Botulinum, J. Infect. Dis. **35**:576, 1925.

12. Coleman, G. F.: Intramuscular Infection of Guinea Pigs with Spore of Clostridium Botulinum, Am. J. Hyg. **9**:46, 1929.

13. Wynne, E. S., and Foster, J. W.: Physiological Studies on Spore Germination with Special Reference to Clostridium Botulinum, J. Bact. **55**:61, 1948.

certain species of Clostridium from mixed cultures has recently been reemphasized.[10a] Further, more complete and accurate results are provided by cultivation of débrided tissues from the wound. In Altmeier's [10d] cases the incidence of Clostridium organisms was highest during the first week and progressively decreased with time. The role of the antibiotics can hardly be incriminated, since wound infections are not controlled adequately without complete débridement, and devitalized tissues such as muscle and sequestrums are not sterilized by penicillin.[14]

It may be that failure of clinical recognition of cases accounts for the lack of reported instances. A review of cases of "atypical tetanus," however, reported from World War I [15] did not reveal that the symptoms were suggestive of botulism in any instance.

Apparently only a small percentage of individuals harboring Clostridium organisms present signs of toxicity; e. g., Pulaski [2a] noted no clinical evidence of tetanus or gas gangrene in his series of 200 cases despite an incidence of these organisms of 23 per cent on culture. In addition, under certain in vitro conditions not only do Clostridium sporogenes and other anaerobic species interfere with the production of the toxin of Cl. botulinum but the toxin may be destroyed when incubated with cultures of these organisms.[16]

In the four cases in which Cl. botulinum organisms were reported to have been isolated from the wound, there was no clinical evidence of botulism. In one case Pulaski [2a] gave no details. In two of Hall's [2b] three reported cases the organism was cultured from débrided tissue, with the wounds subsequently healing by first intention. In the third case Cl. botulinum type B organisms were isolated from a thigh wound which was associated with a compound fracture of the femur. The organism was isolated periodically until the forty-second day following the injury, yet there were no symptoms of botulism. That botulism toxin can be absorbed readily from mucous surfaces, broken skin and fresh wounds was shown by Geiger [17] in his experiments on guinea pigs.

COMMENT

Other diagnoses were entertained prior to autopsy and bacteriological studies, viz., poliomyelitis, diphtheria, tetanus, methyl alcohol intoxication, heavy metal poisoning, plant alkaloid poisoning, rabies, encephalitis lethargica, progressive bulbar palsy and several others. No evidence could be obtained to support any of these any more strongly than to keep them in the realm of unlikely possibilities. Botulism was included in this last large group of possible diagnoses. Although the picture presented was one of diffuse muscular paralysis, the manifestations of Cl. botulinum poisoning, this diagnosis could not be correlated with the presence of the thigh wound. It was only after Cl. botulinum, type A, had been isolated from tissue taken from the wound and identified by specific neutralization tests that the

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diagnosis of botulism was established. Had this diagnosis been seriously considered before the death of the patient, an attempt to demonstrate the toxin in the blood and the tissues would have been of diagnostic aid.[18]

SUMMARY

A case of fatal botulism due to Cl. botulinum infection of a relatively minor gunshot wound of the thigh of a 13 year old boy is presented. The patient died approximately nine days after the injury.

The disease followed the course of a rapidly progressive generalized muscular weakness and great prostration, associated with dysphagia, dysphonia and inability to cough. Death was due to respiratory failure and bilateral lobular pneumonia. This case is of unusual interest, since the literature contains no previous reports of cases in which botulism occurred following Cl. botulinum infection of a wound. Despite the absence of previous reports, it would seem that botulism from this source might occur more frequently. The organism is ubiquitous in nature, a potential contaminant of every traumatic wound. Furthermore, its growth requirements for initiating an infection appear to be practically identical with those of Cl. tetani. Finally, botulism has been produced in experimental animals by the injection of heated spores, and investigators have demonstrated that the botulinus toxin may be absorbed from broken skin and fresh wounds.

The observations in this case are consistent with the findings in botulism. The severe cerebral edema is a feature that has not been previously commented on. The most reasonable explanation is that it is not a manifestation of the botulism per se but is a consequence of the respiratory failure and cerebral hypoxia.

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LOCALIZATION OF THE NEPHROTOXIC ANTIGEN WITHIN THE ISOLATED RENAL GLOMERULUS

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IT IS WELL established that the kidney contains a specific antigen, the antiserum of which is nephrotoxic and capable of producing glomerulonephritis with striking similarities to the spontaneous disease in man. Pearce [1] showed, many years ago, that this antigen resided in the renal cortex and not in the medulla, a finding reaffirmed by Heymann, Gilkey and Salehar.[2] Recent studies, however, have further localized this antigen within the cortex to the renal glomerulus. Pressman and his co-workers,[3] employing intravenous injections of radioiodinated globulin prepared from rabbit antimouse nephrotoxic serum, have demonstrated the early localization, rise in concentration and prolonged persistence of radioactivity in mouse kidney tissue and specifically, by means of autographs, the concentration of radioactivity within glomeruli. Solomon, Gardella, Fanger, Dethier and Ferrebee [4] isolated rat renal glomeruli by means of a modified Potter-Elvehjem homogenizer and showed that the potency of rabbit antirat nephrotoxic serum could be nullified by adsorbing this serum with the isolated glomeruli. Greenspon and Krakower [5] presented a simple procedure for obtaining large numbers of isolated glomeruli from the renal cortex of dogs and demonstrated that approximately 400,000 isolated glomeruli were capable of producing a potent nephrotoxic serum when inoculated into rabbits, while all other components of the cortex were incapable of doing so.

The present study concerns itself with the site of the antigen within the isolated glomerulus.

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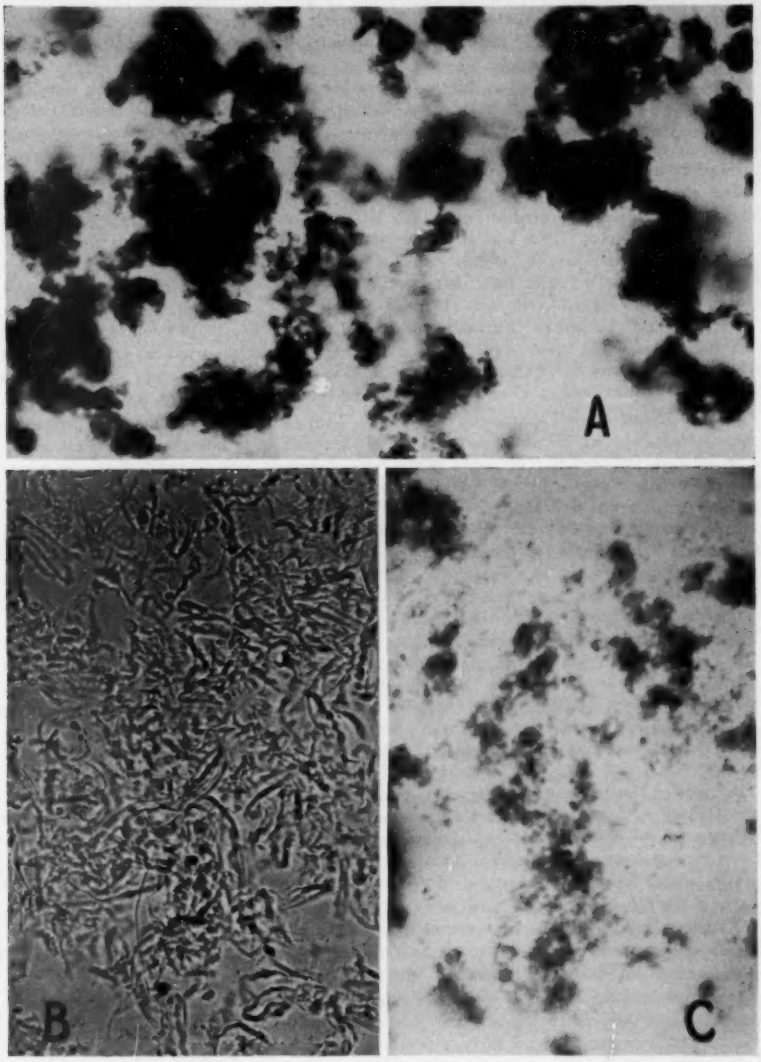
PROCEDURES AND METHODS

Kidneys were obtained from dogs under sterile conditions and perfused with sterile isotonic sodium chloride solution. They were stored immediately at —25 C. Eighteen kidneys, as a rule, were processed at one time in a sterile room equipped with ultraviolet lamps. All equipment was sterile, and all material was handled with sterilized rubber gloves. The kidneys were taken out of the deep freeze the night before and placed in the coldest compartment of the refrigerator overnight to allow for slow thawing. They were still quite frozen when used. The medullae were rapidly dissected away from the kidneys, and the cortices were pushed through a 150 mesh monel metal sieve with an angulated spatula. The kidney mash, made up of cellular components of the tubules, glomeruli, free parietal glomerular capsules and a variable number of short tubular segments, was received into an ice-cold container. Isotonic sodium chloride solution was added to this with stirring, and the suspended material was transferred to test tubes kept in a bath of ice. Each kidney was processed separately, a clean sieve being used for each kidney. The sieves were scoured with a brush, placed in cleaning solution and then sterilized in a liquid disinfectant. They were rinsed in sterile water or saline solution prior to use. Each test tube containing the kidney suspension was spun at 1,500 revolutions per minute (rpm) for about five minutes. The supernatant was briskly removed by suction. The glomeruli were resuspended in saline solution and allowed to settle, and the supernatant was again drawn off by suction. This was repeated several times until the glomeruli were as clean of contaminants as possible. The tubes were at all times kept in a bath of floating ice. Suspending the kidney mash in 82 per cent sucrose solution as originally described [5] was found to be unnecessary and was abandoned in later experiments. The contents of each tube were examined microscopically, regard being paid to the number of glomeruli with and without parietal capsules and the number of tubular segments. In all instances the cellular debris from the renal tubules was completely eliminated. In some preparations, however, there were substantial numbers of tubular segments. These specimens were discarded. Only preparations with minimal numbers of short tubular segments were used. Tubes containing a high percentage of encapsulated glomeruli were pooled, as were those with glomeruli few of which retained their parietal capsules. The pooled glomeruli were spun at 1,500 rpm for five to 10 minutes and firmly packed, yielding as a rule a firm, fawnish gray, translucent mass. The packed wet glomeruli were weighed. They were then suspended in 15 cc. of isotonic sodium chloride solution. The numbers of glomeruli, tubular segments and free parietal capsules were determined by means of a hemacytometer. The percentage of glomeruli which had retained their parietal capsules was similarly estimated. Samples of whole glomeruli were tested for their antigenicity.

Two Minute Sonic Vibration of Glomeruli.—The vibrator used was of a type operating on the magnetostriction oscillator principle [6] and cooled by running tap water. In order to liberate cells from glomeruli, the vibrator was operated at a plate and output voltage of 20 volts for two minutes. Further vibration at this voltage greatly distorted the nuclei of the cells and yielded in all an inappreciable number of additional cells. The 15 cc. of suspended glomeruli were removed from the vibrator after two minutes and allowed to settle in a conical test tube or spun at 800 rpm for a few minutes. The descent of the heavier glomeruli could be readily followed with the naked eye. When the supernatant appeared to be free of glomeruli, this opalescent fluid was withdrawn by means of a pipet. The supernatant fluid microscopically was found to be strikingly free of glomeruli but contained numerous cells.

The cells varied considerably in shape and to some extent in size. They contained well defined, largely undistorted nuclei with surrounding refractile granules held together by a cytoplasmic film. There were no well defined cytoplasmic borders, however. It was felt that the larger cells with more abundant granules were probably of visceral epithelial origin (*A* in figure). These were predominant in the mount. The smaller cells, with little cytoplasm and few granules, were thought to be of endothelial origin. The quality of these cells was in keeping with that of fresh mounts of glomeruli of which studies were made. Well defined, refractile granules were always found within the relatively abundant cytoplasm of visceral epithelial cells. Such granules, however, were few within the small amount of perinuclear cytoplasm of endothelial cells. These

6. Magnetostriction Oscillator Model S-102, 50 watt, 9 kilocycles. Raytheon Mfg. Company, Waltham, Mass.



A, isolated glomerular cells, predominantly visceral epithelium, stained with methylene blue; × 800.

B, unstained mount of basement membranes and parietal capsules from 20 minute sonically vibrated glomeruli; × 370.

C, unstained high speed sediment of 20 minute sonically vibrated glomeruli, made up principally of nuclear particles and cytoplasmic granules; × 850.

granules appeared more numerous and larger when the glomeruli were allowed to autolyze even slightly. Supravital staining of glomeruli from freshly killed dogs revealed some uptake of Janus green and neutral red in the cytoplasmic granules, indicating that some of these at least were of mitochondrial character.

With this amount of vibration, few cells were liberated from parietal capsules, since these were not separated from the glomeruli, and only those still adherent to free parietal capsules might be. As a rule, the cells of the free parietal capsules were largely washed away during the process of cleaning the glomeruli. The occasional tubular epithelial cell could readily be identified by its large size, larger nucleus and more abundant mitochondrial granules.

The glomeruli that had settled were fully decellulated in some instances, partly in other instances, while many appeared to retain their full complement of cells, particularly those with intact parietal capsules. There was no fragmentation of tufts or loops. Attempts were made to determine the relative loss of visceral epithelial and endothelial cells from glomeruli prior to and after vibration—but with little success. We have always found it difficult to distinguish between these two types of cells with precision.

The cells were packed by centrifugation at 1,500 rpm for five to 10 minutes, washed three times and weighed. The supernatant, after the cells had been packed, was still somewhat opalescent. The antigenicity of this supernatant, as well as that of the cells, was determined. The material at all times, except when being vibrated or centrifuged, was kept cold.

Twenty Minute Sonic Vibration of Glomeruli.—With the vibrator operated at full plate and output voltage, viz., at 130 volts, unencapsulated glomeruli were immediately decellulated and the loops were fragmented. The nuclei of the cells elongated, became distorted and soon were fragmented. Those glomeruli with parietal capsules were momentarily resistant to vibration; very shortly, however, the capsules were stripped away from the glomeruli. The latter, in turn, were decellulated, and both the parietal capsules and basement membranes of the loops were fragmented. It was found by repeated trials that a 20 minute period of vibration was optimal. At this point, microscopically, there were no intact glomeruli but only clean, translucent, refractile plates, optically free of any trace of cell (*B* in figure). The thicker, larger plates could be readily identified as derived from the parietal capsule, while the thinner plates with portions of a spiral or looping configuration clearly originated from basement membranes of the glomerular tufts. This suspension was packed by centrifugation at 1,500 rpm for five to 10 minutes, yielding a firm, gray, translucent sediment. In addition to the clean, clear, refractile plates described above, this sediment contained microscopically visible fine particles and some larger granules. These particles had an affinity for methylene blue and presumably were nuclear material. Prior to use, the sediment was washed three times, and this greatly reduced the numbers of nuclear particles.

Portions of this sediment were fixed, sectioned and stained in a variety of ways. The clear plates stained red with Hotchkiss' periodic acid-acid fuchsin; blue with Masson's trichrome stain, Mallory's aniline blue, and Mallory-Heidenhain azocarmine; red with hematoxylin-eosin and iron-hematoxylin-eosin. They stained pale pink to red with Van Gieson's stain and failed to stain for elastic tissue by the method of Weigert or for reticulum by the method of Foot. In none of these sections were intact nuclei or cells identifiable. Rare scattered blackish particles were observed in the sections stained with iron-hematoxylin-eosin.

This sediment generally formed 40 to 50 per cent by weight of the original glomerular sample, independent of the percentage of parietal capsules.

The supernatant of 20 minute vibrated glomeruli was quite opalescent after slow centrifugation. It was further centrifuged at 10,000 to 15,000 rpm for 15 minutes. A sediment was now obtained which was gray with a variable degree of brown coloration that was spotty or diffuse. Microscopically, this sediment was composed of granules and threads of coccobacillary proportions which stained deeply with methylene blue (*C* in figure). When fixed, sectioned and stained, some of the threads were argentophilic, while the granules had an affinity to some extent for iron-hematoxylin and the azure of Giemsa's stain. These particles and threads, however, failed to stain in the characteristic way in which the fragments of basement membrane and parietal capsule did, as indicated above. This fraction appeared to contain four components. The two predominant components were nuclear fragments and cytoplasmic granules. The latter were derived principally from the visceral epithelial cells but to some extent also from parietal cap-

sular epithelium and endothelium of the capillary loops. The argentophilic threads probably in part originated from the outer portions of the parietal capsule. Some of the threads and particles undoubtedly were derived from basement membrane. From estimates of the amount of material lost to high speed sediment and supernatant after two hours of sonic vibration of parietal capsules and basement membrane, it is reasonable to conclude that approximately 3 to 5 per cent of the latter structures would be lost in a 20 minute vibration. The percentage was probably higher, since more material would be lost during the initial vibration when the cells were being shorn away from their framework.

The sediment obtained from high speed centrifugation formed about 19 per cent of the initial total weight of the glomeruli when the parietal capsular content was low and close to 25 per cent when it was high.

The supernatant, following high speed centrifugation, was fairly opalescent. It contained from 25 to 40 per cent of the original glomerular material, varying with the percentage of encapsulated glomeruli. No granules or particles could be seen in this preparation under the highest power of the light microscope. It was assumed that this fraction contained nuclear material, cellular cytoplasm both in its soluble and in its small granule form (at least granules nonsedimentable when centrifuged at 10,000 to 15,000 rpm for the given length of time), and some material from glomerular membranes.

The high speed sediment was washed three times and weighed prior to being inoculated into rabbits. All fractions were kept cold until ready to be used.

Chemical Analyses.—Nitrogen determinations on whole glomeruli, cells, sediments and supernatants were performed according to the micro-Kjeldahl procedure of Ma and Zuazaga.[7] The ammonia was absorbed in boric acid according to Winkler's[8] procedure.

These materials were also prepared by Schneider's method[9] for the determination of nucleic acids. The quantity of desoxypentose was estimated by the spectrophotometric method of Dische,[10] using diphenylamine. Pentoses were determined by the Bial test or the orcinol method of Mejbaum.[11]

Preparation of Material for Inoculation and Procedure of Inoculation.—Cells and sediments were weighed and the amounts of material lost to the supernatants determined by difference. All sediments were made up to a given volume so that, on the average, each milliliter contained 5 or 10 mg. when smaller amounts were being used. Amounts greater than 75 mg. were weighed out directly. The individual suspensions were mixed with aluminum hydroxide jelly prepared by the method of Tracy and Welker.[12] Equal parts of suspension and jelly were generally used, so that the supernatant solution obtained by centrifugation gave a negative biuret test. Equal parts of supernatant and jelly were mixed without further testing. Rabbits weighing 2,500 Gm. were inoculated in each thigh with varying amounts of this material. They were bled from the heart at the end of three weeks. The serum was stored at —25 C. Serum from each rabbit was tested separately, even though the character and the amount of material inoculated into the rabbits had been the same. Healthy dogs kept in metabolism cages were inoculated intravenously with 1.5 ml. of serum per pound after their urines had been found to be free of abnormal constituents on two consecutive days. Urines were collected on the fifth, sixth and seventh days after inoculation and tested for albumin qualitatively and quantitatively (by means of a photoelectric colorimeter) with Exton's[13] reagent, containing bromophenol blue. The dogs were killed on the seventh day. Complete autopsies were performed. In all instances, sections of the kidneys were prepared for microscopic study. In many instances, sections of the other organs were processed routinely.

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RESULTS

In general, 18 dog kidneys yielded approximately 11 million glomeruli. In eight preparations in which 19 per cent or less of the glomeruli retained their parietal capsules, the average wet weight per glomerulus was 0.21 microgram, with a range of 0.13 to 0.30, whereas in 11 preparations with 50 per cent or more encapsulated glomeruli, the average wet weight per glomerulus was 0.26 microgram, with a range of 0.17 to 0.36. It can be determined from these figures that each parietal capsule contributed approximately 0.16 microgram to the whole weight of the glomerulus and that the bare glomerulus weighed approximately 0.18 microgram.

Free parietal capsules averaged 1.2 per cent of the total glomerular count. In 18 of 25 preparations the number of free parietal capsules was 1 per cent or less of the total glomerular count. In the remaining 7 it exceeded 1 per cent, with a maximum of 5.2 per cent.

The short tubular segments present in the preparations were similar in size to glomeruli, though more threadlike in shape. Presumably they were of about the same density as the glomeruli, since they settled with the same speed as the glomeruli proper. These small segments numbered 0.5 per cent or less of the total glomerular count in 12 of 25 preparations; between 0.5 and 1.5 per cent in seven, and greater than 1.5 per cent but less than 8.2 per cent in the remaining six preparations. The average figure was 1.7 per cent. In view of previous experiments [5] in which all components of the renal cortex except the glomeruli were found to be nonantigenic, this exceedingly low percentage of contaminant can be dismissed as a possible source of antigen in the present preparations.

A serum was regarded as positively nephrotoxic when the dog had albumin (1 + or more) (approximately 100 mg. per 100 cc.) in the urine and showed glomerular lesions microscopically, even when these were spotty or slight. In most instances, however, the urinary albumin ranged from 200 to 400 mg. per 100 cc., and the histologic lesions of the kidneys were of diffuse and characteristic proliferative and hemorrhagic glomerular type.

Antigenicity of Whole Glomeruli.—Two types of preparations were compared. The one with 19 per cent or less encapsulated glomeruli was considered as representative of glomeruli with a low content of intact parietal capsules. The lowest percentage obtainable in this series was 7.5. The other type of preparation contained 50 per cent or more encapsulated glomeruli, with a maximum of 72 per cent.

It was found that whole glomeruli low in parietal capsules were capable of producing a positive nephrotoxic serum in amounts between 15 and 20 mg. or 0.05 and 0.07 mg. nitrogen, whereas with whole glomeruli high in parietal capsules the amount of material required to produce an effective nephrotoxic serum was between 20 and 25 mg. or 0.22 and 0.28 mg. nitrogen (table 1).

This was indicative of the relative absence of antigen in the parietal capsules, a finding that was supported experimentally on using basement membrane and parietal capsules derived from 20 minute sonically vibrated glomeruli.

Antigenicity of the Fractions of Glomeruli Obtained by 20 Minute Sonic Vibration of Whole Glomeruli.—It is quite clear from table 2 that the glomerular basement membranes with a lesser number of parietal capsules were effective in the production of nephrotoxic serums at a level between 5 and 10 mg. or 0.02 and 0.04

mg. nitrogen. On the other hand, glomerular basement membranes with a high content of parietal capsules were not fully active at 15 mg. and attained fuller activity only at 20 mg. Roughly, however, it may be stated that a certain amount of effective antigenicity occurred within the range of 10 to 15 mg. or 0.12 to 0.19 mg. nitrogen.

This is substantial evidence for the absence of antigenicity of the parietal capsules.

The antigenicity of the high speed centrifugate made up predominantly of nuclear particles and cellular granules was erratic. Of the six positive serums listed (table 3), only two were strongly positive. The others were weak.

TABLE 1.—*Comparison of Antigenicity of Serums from Whole Glomeruli with Low and High Contents of Parietal Capsules*

Source of Antigen	Content of Nitrogen, Desoxypentosenucleic Acid and Pentosenucleic Acid, Mg. per Gm. of Wet Weight			Production of Nephrotoxic Serums with Given Amount of Material (Mg.) Inoculated into Rabbits *						
	N	DNA	PNA	5	10	15	20	25	30	35
Whole glomeruli with 19% or less of parietal capsules	2.4	7.5	5.0	.....	0 (4)	1 (4)	3 (4)	.....	.....	.....
Whole glomeruli with 50% or more of parietal capsules	11.2	9.0	20.0	.....	0 (2)	.....	0 (3)	4 (4)	3 (3)	1 (1)

* The number of positively nephrotoxic serums is compared with the number of serums tested in dogs (represented by the figure in parentheses).

TABLE 2.—*Comparison of Antigenicity of Serums of Parietal Capsules and Basement Membranes from Twenty Minute Sonically Vibrated Glomeruli*

Source of Antigen	Content of Nitrogen, Desoxypentosenucleic Acid and Pentosenucleic Acid, Mg. per Gm. of Wet Weight			Production of Nephrotoxic Serums with Given Amount of Material (Mg.) Inoculated into Rabbits *								
	N	DNA	PNA	5	10	15	20	25	30	35	50	75
Basement membrane with low content of parietal capsules	4.4	15.7	2.9	1 (4)	4 (5)	2 (2)	.....	2 (2)	.....	1 (1)	1 (1)	1 (1)
Basement membrane with high content of parietal capsules	12.4	11.2	12.9	0 (1)	0 (3)	3 (6)	4 (5)	4 (4)	.....	.....	4 (4)	1 (1)

* The number of positively nephrotoxic serums is compared with the number of serums tested in dogs (represented by the figure in parentheses).

The antigenicity of the supernatant of the high speed centrifugate made up predominantly of cellular components of soluble and small granule form was quite inferior to that of basement membrane and lay within the range of 125 to 150 mg. or 1.5 to 1.7 mg. nitrogen (table 3).

The much higher content of desoxypentosenucleic acid (DNA) and pentosenucleic acid (PNA) in the high speed sediment and supernatant fractions as compared with basement membrane, despite the lesser antigenicity of the former two fractions as compared with the latter, would tend to exclude nuclear and cytoplasmic nucleic acids or nucleoproteins as nephrotoxic agents. Further evidence in support of this conclusion will be given below, in the discussion of isolated glomerular cells and their supernatant.

Since the high speed sediment and supernatant of 20 minute sonically vibrated glomeruli represented in the main the cellular components of the glomeruli, it was deemed wise to compare the antigenicity of these with that of isolated glomerular cells.

Antigenicity of Isolated Glomerular Cells.—As can be seen from table 3, the effective antigenicity of the cells ranged between 100 and 125 mg. The two serums tested at 100 mg. were very weakly positive. The three serums tested at 125 mg. or the equivalent of 0.65 mg. nitrogen were, however, all strongly positive.

In the process of liberating the cells, cytoplasmic and nuclear material was set free into the supernatant solution. The latter, however, was not further centrifuged at high speed—after the cells had been packed—and hence included larger granules. One hundred and fifty milligrams of this supernatant or the equivalent of 2.5 mg. nitrogen was strongly nephrotoxic.

TABLE 3.—*Comparison of Antigenicity of High Speed Sediment and Supernatant of Twenty Minute Sonically Vibrated Glomeruli Representing All the Cells of the Glomerulus with Isolated Glomerular Cells of Predominantly Visceral Epithelial Origin* *

Source of Antigen	Content of Nitrogen, Desoxypentosenucleic Acid and Pentosenucleic Acid, Mg. per Gm. of Wet Weight			Production of Nephrotoxic Serums with Given Amount of Material (Mg.) Inoculated into Rabbits †								
	N	DNA	PNA	25	30	35	50	75	100	125	150	175
High speed centrifugate.....	10.5	28.5	21.4	0 (2)	.....	.....	0 (3)	0 (4)	4 (6)	2 (4)	0 (1)	0 (1)
High speed supernatant.....	11.7	28.0	31.0	0 (5)	.....	.....	0 (6)	1 (5)	1 (3)	2 (5)	1 (1)	.....
Isolated glomerular cells....	5.2	11.0	27.7	.....	.....	.....	.....	1 (2)	2 (4)	3 (3)	.....	.....
Supernatant of isolated glomerular cells	17.0	47.8	59.8	.....	.....	.....	0 (2)	0 (1)	.....	.....	2 (2)	.....

* In order to obtain an adequate amount of 20 minute vibrated, high speed sediment and supernatant for inoculation, it was necessary at times to combine the yields of sediment from preparations with both low and high percentage of capsules or use the total mixed lot of glomeruli for supernatants. It is for this reason that the results were combined.

† The number of positively nephrotoxic serums is compared with the number of serums tested in dogs (represented by the figure in parentheses).

The somewhat similar antigenic activity of this supernatant as contrasted with isolated cells, despite its apparent greater content of nucleic acids, would again support the view that nucleoproteins play little, if any, role as nephrotoxic antigens.

It will be noted that the antigenic titers of isolated cells and supernatant were sharp and well defined, whereas those of the high speed sediment and supernatant of 20 minute vibrated glomeruli were erratic, although more or less within the same range. There was, however, very good agreement in nitrogen and nucleic acid contents of these combined samples. The difference between them was that isolated cells and supernatant were apparently of predominantly visceral epithelial origin, while the 20 minute-vibrated, high speed sediment and supernatant contained all the cells of the glomerulus, including those of the parietal capsule. It is very likely that the additional parietal capsular cells were in part instrumental in reducing the antigenicity of the vibrated fractions, indicating that these cells—even as the parietal capsule proper—were not antigenic.

In our approximate estimation of the proportion of visceral epithelial cells to endothelial cells in the glomeruli of normal dogs, the former have rarely exceeded the latter above a 2:1 or 3:1 ratio. This ratio would appear to have been much higher in the samples of isolated cells. It would seem, therefore, that the amount

of antigenic material associated with the cells or their fractions is not too dependent on either of these two cell types.

It is logical to assume that if the visceral epithelial and endothelial cells of the glomerulus are the main source and depot of antigen, their nephrotoxic activity would have exceeded that of the basement membrane. That not being the case, the problem therefore remains whether the antigen is distributed in the basement membrane or in films of cytoplasm, optically not apparent, which cover the basement membrane. It is essential to bear in mind that the structure of the glomerulus is not fully known. There is, for example, no agreement as to whether the visceral epithelial cells fully clothe the basement membrane except perhaps at contact points of the loops or whether they are more pericytic in character, with multiple cytoplasmic processes leaving relatively wide areas of basement membrane free of any cellular component on its capsular surface. Likewise, it is not established that there is a complete endothelial lining. Cell boundaries outlined by silver stains, as exemplified for many ordinary capillaries, have not been demonstrated in the glomerulus. There is a growing belief, in fact, that the endothelial cells do not form a continuous layer (von Möllendorff [14]; Bell [15]). That films of visceral epithelium would remain attached to the basement membrane following vibration in such quantity as to account for the greater antigenicity of this structure seems very unlikely, since each visceral epithelial cell had an abundant amount of cytoplasm and represented the predominant cell type. However, the fewer endothelial cells and their small amount of cytoplasm require closer scrutiny with regard to the possibility that they may be the bearers of antigen, since if there are continuous endothelial cytoplasmic films, the amount of apparent cytoplasm which is totally removed by vibration would form only a very small fraction of that which was left intimately bound to the basement membrane.

It was with this in mind that the following procedures were carried out.

Prolonged Sonic Vibration and Grinding of Glomerular Basement Membranes and Parietal Capsules.—Twenty minute-vibrated, low speed-centrifuged glomerular sediments made up of parietal capsules and glomerular basement membranes were sonically vibrated at 130 volts for two hours. It was hoped thereby that possible endothelial cytoplasmic films might be delaminated and released into the high speed sediment and supernatant. It was found, however, that although the fragments of membranes were considerably reduced in size, their antigenicity remained the same and, in fact, strongly supported the nonantigenicity of the parietal capsules as indicated by the differences in the two types of preparations: one with low and one with high percentage of capsules (table 4). The nitrogen content of the membranes remained more or less the same, while the contents of desoxypentose and pentosenucleic acids were somewhat reduced. The amount of material lost to the high speed sediment and supernatant after two hour vibration approximated 21.0 per cent of the original amount of membranes, 15.0 per cent being accounted for in the high speed sediment. These two fractions still contained a fairly high content of nitrogen, desoxypentosenucleic acid and pentosenucleic acid. In addition to the several

14. von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 7.

15. Bell, E. T.: Renal Diseases, Philadelphia, Lea & Febiger, 1946.

serums derived from injection of these fractions which were tested, serums from the high speed centrifugate and supernatant of glomeruli vibrated continuously for two hours were tested (table 4).

Although there are inadequate data in the lower ranges, it would appear that there was an increase in antigenicity of the material lost to basement membrane and parietal capsule by two-hour vibration over that obtained in these fractions by 20 minute vibration of whole glomeruli. Conversely, there was no loss in antigenic content of the glomerular basement membrane.

Further vibration of the membranes of a preparation with a high percentage of capsules for two additional hours was tried. This reduced the fragments of parietal capsule and basement membrane to very small particles. The material, however, stood up poorly to this prolonged treatment. There was a profound reduction principally in the amount of desoxypentosenucleic acid and also in pentosenucleic acid of the membranes at the end of this period (3.3 mg. and 8.9 mg., respectively). The nitrogen content remained nearly the same. One serum derived from 15 mg.

TABLE 4.—*Two Hour Sonically Vibrated Parietal Capsules and Basement Membranes, and Whole Glomeruli*

	Production of Nephrotoxic Serums with Given Amount of Material (Mg.) Inoculated into Rabbits *											
Source of Antigen	5	10	15	20	25	30	35	50	75	100	150	200
1. (a) Basement membrane with low content of parietal capsules	1 (2)	2 (2)	.....	.....	.....	.....	1 (1)	.....	1 (1)	.....	.....	.....
(b) High speed centrifugate	.....	.....	.....	.....	.....	.....	0 (1)	.....	1 (1)	.....	.....	.....
(c) High speed supernatant	.....	.....	.....	.....	.....	.....	.....	0 (1)	1 (1)	.....	.....	.....
2. (a) Basement membrane with high content of parietal capsules	.....	0 (2)	0 (1)	2 (2)	2 (2)	1 (1)	.....	.....	.....	.....	.....	.....
(b) High speed centrifugate	.....	.....	.....	.....	0 (1)	.....	.....	1 (1)	.....	.....	.....	.....
3. (a) High speed centrifugate of whole glomeruli with low content of parietal capsules	.....	.....	.....	.....	.....	.....	.....	.....	1 (1)	.....	1 (1)	1 (1)
(b) High speed supernatant	.....	.....	.....	.....	.....	.....	.....	0 (2)	.....	1 (1)	.....	1 (1)

* The number of positively nephrotoxic serums is compared with the number of serums tested in dogs (represented by the figure in parentheses).

of the membranes was negative; one serum each from 20 mg., 25 mg. and 35 mg., and two from 75 mg., were all positive. The high speed sediment of the four hour vibrated membranes contained 1.2 mg. of nitrogen, 2.1 mg. of desoxypentosenucleic acid, and 6.2 mg. of pentosenucleic acid. One serum derived from 25 mg. was negative, while two serums from 75 and one from 100 mg. were positive, for nephrotoxic property. The supernatant of the high speed sediment contained 5.0 mg. nitrogen, 12.9 mg. desoxypentosenucleic acid, and 35.8 mg. pentosenucleic acid. Two serums were tested: One derived from 50 mg. was negative, but one from 75 mg. was positive, for nephrotoxic effect.

These data provided further evidence that the increased antigenic activity, particularly of the high speed sediments but also of the supernatants, after two or four hours of vibration was dependent on the reduction in particle size of the basement membrane to the extent that the very small particles were now incorporated in these fractions. There was no evidence for any delamination of an endothelial cytoplasmic film.

Parietal capsules and basement membranes obtained by 20 minute sonic vibration of glomeruli were ground with sand for prolonged periods, again with the

thought that adherent cytoplasmic films might be released. The high speed sediments and supernatants of these preparations both failed to produce nephrotoxic serums. The ground membranes continued to be antigenically active, however.

It seems apparent, therefore, that the antigen is concentrated within the basement membrane which, as here prepared, was optically free of cells. Had we been able to free our samples of all parietal capsules, it is likely that 5 mg. or less (0.02 mg. nitrogen or less) of basement membrane would have been adequate to produce consistently an active nephrotoxic serum. This would represent the combined basement membranes of approximately 50,000 glomeruli. If one combines the antigenic titers of isolated cells and supernatant or of vibrated whole glomerular high speed sediment and supernatant, it would require approximately 100 mg. of cells or 1 mg. of cell nitrogen to produce an active serum. Hence the basement membrane is about 20 times more active than the cells in terms of wet weight or 50 times more active in terms of nitrogen content. In view of the apparent indifference as to cell type tested and of the inability—at least to date—to recognize or separate endothelial cytoplasmic films, the belief is held that the antigen is distributed throughout the basement membrane with active surface components. The latter are apparently carried away with the cells—visceral epithelial and endothelial—when such cells are separated from the basement membrane.

SUMMARY

Isolated renal glomeruli were sonically vibrated, yielding at low voltage isolated cells, predominantly of visceral epithelial origin. At high voltage, three fractions were obtained: a low speed centrifugate made up of clear, refractile plates of parietal capsule and basement membrane, optically free of cells; a high speed centrifugate and supernatant representing most of the components of all the cells of the glomerulus. By studying the antigenicity of whole glomeruli and low speed centrifugates with a high or low percentage of parietal capsules, it could be clearly shown that the parietal capsules were not antigenic. Isolated cells and all the cells of the glomerulus had approximately the same degree of antigenicity, yet with a sufficient degree of difference probably to exclude the parietal epithelial cells as bearers of antigen. By virtue of the distribution of desoxypentose and pentosenucleic acids in the high voltage fractions and isolated cells, nuclei and cytoplasmic nucleoproteins could be eliminated as nephrotoxic antigens. Basement membrane was found to be 20 times more active antigenically than the combined visceral epithelial and endothelial cells of the glomerulus in terms of wet weight and 50 times more active on the basis of nitrogen content. Because of the inability to recognize morphologically or separate experimentally cellular cytoplasmic films which might be adherent to the basement membrane, the view is held that the nephrotoxic antigen is distributed throughout the basement membrane and that surface antigens are released when visceral epithelial or endothelial cells or both cell layers are shorn away.

Case Reports

POLYOSTOTIC FIBROUS DYSPLASIA (ALBRIGHT'S SYNDROME)

Report of a Case Showing Central Nervous System Changes

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ALBRIGHT'S disease (polyostotic fibrous dysplasia or osteitis fibrosa cystica disseminata) is a well defined clinical syndrome characterized by disseminate bone lesions, endocrine disorders and cutaneous pigmentations. Over 40 cases of the disease have been reported in the medical literature, and extensive pathological descriptions of the osseous lesions observed in biopsy material are available. However, little is known concerning pathological changes occurring in other systems, only one report of a complete autopsy having been published.[1] In the report of another autopsy only the gross findings were described.[2] It may serve some useful purpose, therefore, to place on record an additional instance of Albright's disease with the pathological observations. Further interest may be found in the clinical and pathological findings indicating conspicuous involvement of the central nervous system.

REPORT OF CASE

A boy 5 years of age was admitted to Willowbrook State School because of idiocy and epilepsy. He was the only son of unrelated, healthy Jewish parents. There had been no similar disease in any other members of the family. However, none of these was examined personally.

Delivery of the patient was at term, following an uneventful gestation, low forceps being used without apparent injury to the head. The birth weight was 6 lb. 4 oz. (2,835 Gm.). During the first five months of life nothing abnormal was noted in the development of the child. At the age of 5 months, without apparent cause, a series of severe generalized convulsive seizures were observed over a period of a few days. Physical and neurological examinations gave negative results, but a pneumoencephalographic study suggested that a subdural hematoma was present on the right side. Surgical exploration, however, did not disclose one. Severe convulsions continued at irregular intervals, at times of months, at other times of days. Mental deficiency became evident at about 9 months of age and appeared to be of the utmost gravity. The child never spoke, never attempted to stand up or walk, and failed to respond to any but the simplest stimuli. Physically, since the age of 1 year, he had been underdeveloped and underweight. Bony deformities began to be noted at the age of 3 years when bowing of the right femur appeared. No roentgenologic study was made at that time.

On admission, at 5 years of age, he was an emaciated, undersized (3 ft. 5 in. [104 cm.]), underweight (27 lb. [11 Kg.]), idiotic child. The head was slightly microcephalic, measuring 19 in. (48 cm.) in circumference. Both femurs appeared bowed, the right to an extreme degree, almost in a semicircle, the left to a mild degree. The feet were held in eversion.

From the Research Departments of Letchworth Village and Willowbrook State School, New York State Department of Mental Hygiene.

1. Sternberg, W. H., and Joseph, V.: Osteodystrophia Fibrosa Combined with Precocious Puberty and Exophthalmic Goiter: Pathologic Report of Case, Am. J. Dis. Child. **63**:748 (April) 1942.

2. Coleman, M.: Brit. J. Surg. **26**:705, 1939.

On roentgenologic examination the skull (fig. 1*A*) showed several "punched out" areas, indicating considerable thinning of the bone. The right femur (fig. 1*B*) exhibited a strikingly distorted diaphysis with numerous and large areas of bone rarefaction, cystlike in appearance, in the distal half and irregular proliferation of bone in the proximal half. There was evidence of healed fracture in the inferior third. The left femur showed similar but much less advanced lesions (fig. 1*B*). The tibias and the fibulas appeared thinner than normal, but there was no change in structure. The right humerus showed an area of rarefaction similar to that observed in the left femur.

All deciduous teeth present were badly eroded nearly to the gingival crest, but the erupting permanent lower central incisors were in good condition.

The skin was dry and very dark in color. In the right lumbar region there was a small flat area of irregular pigmentation 4 in. (10 cm.) in diameter. In addition, a pigmentary mole, 1 in. (2.5 cm.) in diameter, was observed in the thoracic region along the left axillary line. There were no other lesions of the skin. The hair of the head was black, fine and abundant; the hair line was low on the forehead. A rich growth of silky hair covered temples, cheeks, upper lips and the extensor surfaces of the extremities. The pails were normal in appearance.

The thyroid gland was considerably enlarged, being about twice its normal size. It protruded strikingly on the emaciated neck. The thoracic and abdominal organs showed nothing of significance. The penis and the left testicle appeared normal. The right testicle was retained in the canal.

Neurological examination showed generalized paralysis of all muscles of the extremities, the patient being unable to perform any active limb movements. There was marked diffuse muscular atrophy. The deep reflexes were normal, but a certain degree of muscular hypotonicity was observed. No Babinski sign was noted. Continuous rapid twitching movements of the facial muscles and of the flexor muscles of the upper extremities were noted. Coordination and sensory function could not be tested. The cranial nerves as far as testing could be performed were unimpaired. Pupillary reaction appeared normal. Continuous rolling, slow movements of the eyes were present. There was no exophthalmos.

On psychological examination according to the Gesell developmental test, the maturity level was 3 months with a range between 4 and 20 weeks. The patient's mentality was therefore at the low grade idiot level, with an intelligence quotient below 10.

The blood calcium (10.6 mg. per 100 cc.) and the blood inorganic phosphorus (4.0 mg. per 100 cc.) were within normal limits. The serum phosphatase was not determined. The urine contained a moderate amount of albumin and granular casts.

During the patient's month-long stay in the hospital, the physical and mental conditions did not change significantly. Death resulted from bilateral bronchopneumonia.

Autopsy (six hours after death).—Apart from terminal bronchopneumonia the pertinent gross pathological changes included lesions of bones, endocrine glands and the central nervous system.

The osseous lesions involved the femurs and the skull. The right femur was bent in a semicircle with the convexity outward and appeared flattened out, the thickness of the shaft being about 1 cm., the width 5 cm. Cross section of the diaphysis showed irregularly distributed areas of fibrous tissue within the bone. The cortex appeared much thinner than normal. The epiphyses were grossly normal. The left femur was somewhat bent, and the distal part of the diaphysis was softer than normal. The tibias and fibulas were thinner than normal but showed no gross alterations of internal structure. The parietal and frontal bones when inspected against the light showed four translucid areas oval in configuration, about 1 in. (2.5 cm.) in diameter. In these areas the bone was thinner than normal and no spongiosa was present.

Gross lesions of the endocrine glands were limited to the thyroid, which was clearly enlarged, protruding conspicuously under the skin. It measured 2½ by 3 in.

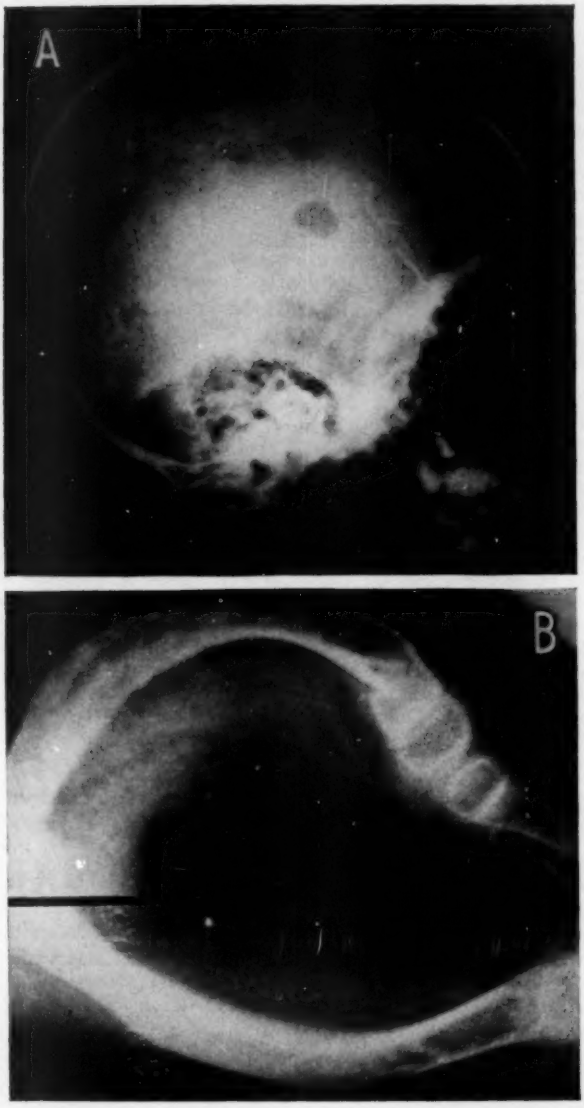

Fig. 1.—*A*, roentgenogram of the skull revealing punched-out areas of thinning bone. B, roentgenograms of the femurs (right above, left below), showing bowing and cystlike rarefaction of bone.

(6 by 7.5 cm.) and weighed 29 Gm. Its shape was normal, but its consistency was soft. The parathyroids were grossly normal and not enlarged. The pituitary and the adrenal glands were not grossly different from the normal.

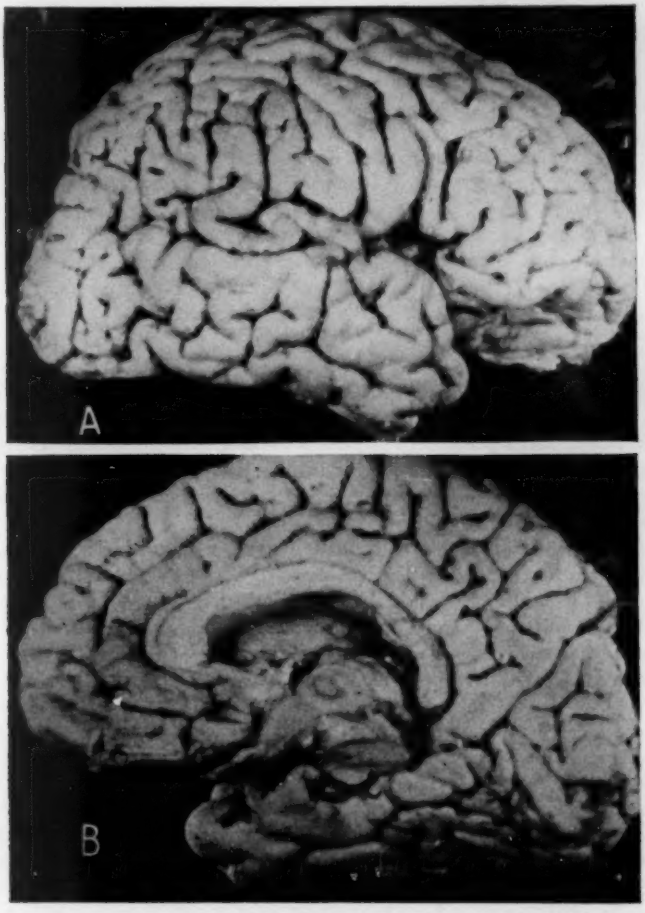

Fig. 2.—External aspects of the brain, showing mild atrophy more pronounced in certain gyri.

The brain weighed 850 Gm. and appeared grossly atrophic (fig. 2). The atrophy was more pronounced in the frontal and temporal lobes than elsewhere. Some convolutions, the first temporal and the third frontal, appeared much smaller than normal, but all main sulci and convolutions could be identified without difficulty. The lateral ventricles were slightly dilated. The cerebellum was somewhat reduced in volume. Midbrain, pons and medulla appeared grossly normal.

Histological Examination.—Changes were observed in the bones, the endocrine glands and the central nervous system. There were no relevant changes in the liver, the kidneys, the pancreas or the heart. In the lungs evidence of recent bronchopneumonia was found. The spleen was normal histologically except for occasional nests of large cells, irregularly polygonal in shape, exhibiting pale vesicular nuclei and large amounts of pale cytoplasm.

The osseous lesions were studied in blocks obtained from the affected areas of the right femur, decalcified with nitric acid and stained with hematoxylin and eosin. The normal structure of the bone was altered by the presence of areas of connective tissue showing delicate fibers and elongated nuclei, loosely arranged (fig. 3), occasionally conferring on the tissue a myxomatous aspect. Vascularization of the connective tissue was poor, and vessels showed thin walls. No hemorrhages were seen. Osteoblasts were observed lying against the bone at the periphery of the connective tissue area. Osteoclasts were not seen. Osseous spicules were present in the connective tissue. No foamy elements or giant cells were observed. No newformed cartilaginous tissue was present in any of the sections examined.

The areas of rarefaction of the skull showed no fibrous dysplasia but only considerable thinning of normal bone tissue.

Of the endocrine glands, the pituitary, of normal size and volume, showed no conspicuous alterations of normal cellular arrangement. Sections at various levels stained with Mallory's phloxine-methylene blue and Mallory's connective tissue stains demonstrated a percentage of various cellular elements within normal limits. Basophilic cells showed no nuclear abnormalities, nor was basophilic adenoma noted. Colloid changes of Crooke were not observed. A few small nests of basophilic cells were observed in the posterior lobe.

The enlarged thyroid gland showed striking changes. The vesicles were enlarged, often to an extreme degree; they were irregular in size and lined by a cuboidal or flat epithelium which showed no infolding (fig. 4). The colloid was often broken down into bandlike fragments after embedding. It stained pinkish in hematoxylin-eosin preparations and orange in Masson's trichrome stain. The interacinar connective tissue was neither increased in amount nor infiltrated. No adenomas of the fetal type were observed. Occasionally, some vesicles contained round or oval cells of various size with an eccentric nucleus, the apparent result of desquamation from the acinar wall. No areas of degeneration and no hemorrhages were observed.

The parathyroids, two of which were embedded in the lower portion of the thyroid, were of normal aspect and size. There were no significant changes. No adenomatous formations were seen, and there were no oxyphilic cells.

The adrenal glands were normal on routine examination of sections stained with hematoxylin and eosin.

The testicles were not examined histologically.

The central nervous system was investigated with the usual methods of neuropathological technique. Sections of cortical fields from various regions prepared according to Nissl's method showed disarrangement of normal cytoarchitecture. Nerve cells were decreased in number without corresponding increase in number of glia nuclei. Individual cells were often distorted, pyramidal cells being oriented in an oblique or transverse direction instead of vertically. Fusiform neurons with some resemblance to neuroblasts were occasionally observed. A certain number of

Fig. 3.—Low power magnification of a section of bone, showing destruction and connective tissue replacement of the bone; hematoxylin and eosin.

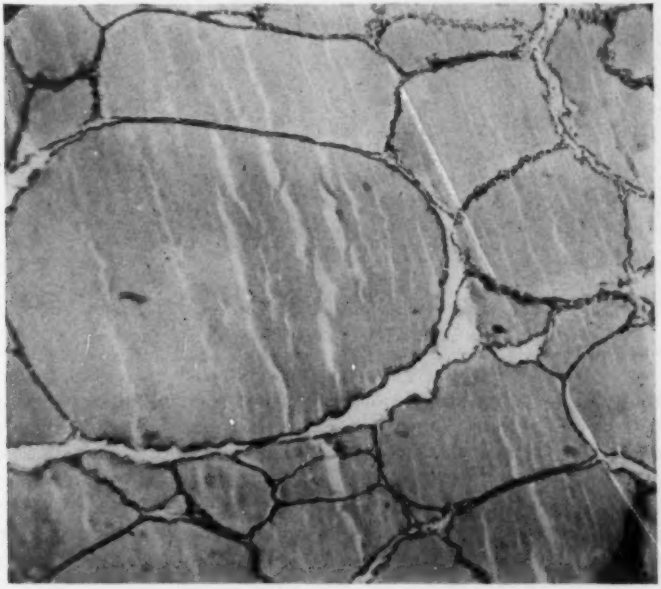

Fig. 4.—Low power magnification of the thyroid gland, showing changes characteristic of colloid goiters; hematoxylin and eosin.

abnormally large cells (fig. 5), obviously of the neurocytic type, well demonstrated by both Nissl and Bodian methods, were observed, some exhibiting pyramidal form, others distorted in shape with bizarrely arranged dendrites. These giant cells were usually isolated or, rarely, collected in small clusters. They were found scattered irregularly in all lobes and in every layer. In appearance they resembled the abnormal giant cells which are considered pathognomonic of tuberous sclerosis. However, in no place were the characteristic cortical glia nodules of tuberous sclerosis

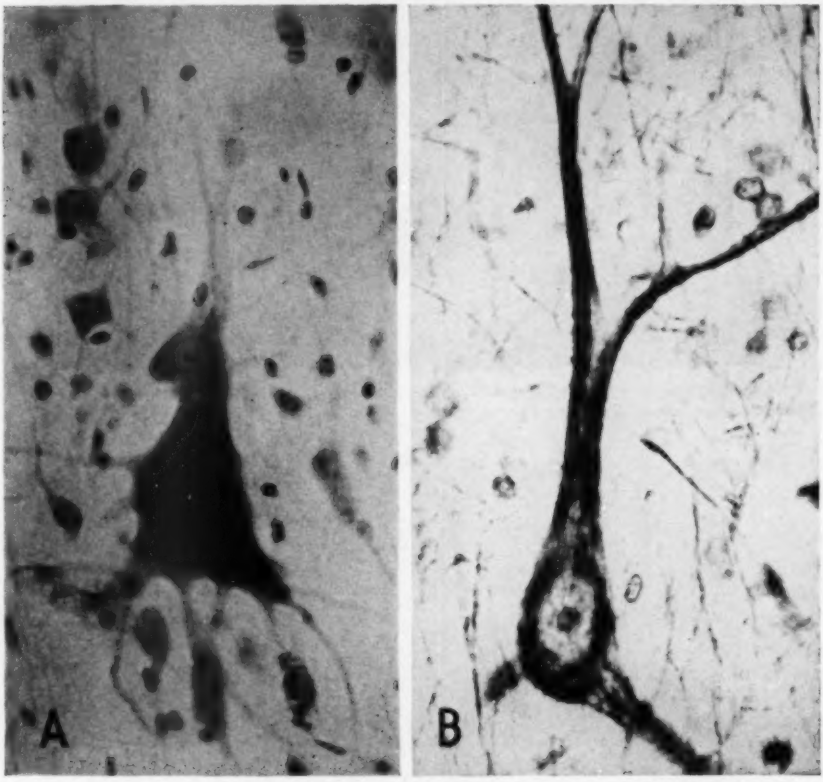

Fig. 5.—Giant cells of the cerebral cortex. Nissl's thionine stain (*A*) and Bodian's strong protein silver (protargol®) impregnation (*B*); high power magnification.

observed, nor were the peculiar "candleguttering" lesions seen in the periventricular regions.

There was no abnormal amount of calcium, and in silver preparations no argentophilic plaques were seen. With myelin stain no changes were present throughout the hemispheres. In preparations stained with scarlet red there was no fatty material within the nerve cells but a certain number of compound granular elements laden with fat were seen in the perivascular space throughout the white matter. Microglia showed no proliferation or hypertrophy. Oligoglia was acutely swollen throughout.

In the basal ganglions no conspicuous lesions were demonstrated in cellular or myelin preparations. In the globus pallidus, small globules of amorphous material taking both fat and iron stains were observed. Numerous sections throughout the thalamus and the hypothalamus were prepared with myelin and cellular stains. Although no study of serial sections was made, the general structure of this region appeared unchanged.

The cerebellum showed peculiar changes involving mainly the layer of Purkinje cells. These were conspicuously diminished in number, leaving many empty spaces in which Bergmann's fibers were not proliferated. Many of the remaining cells were distorted in shape, some polygonal, others fusiform, and irregular in size, at times larger than normal. Occasionally cells with two nuclei were observed. Den-

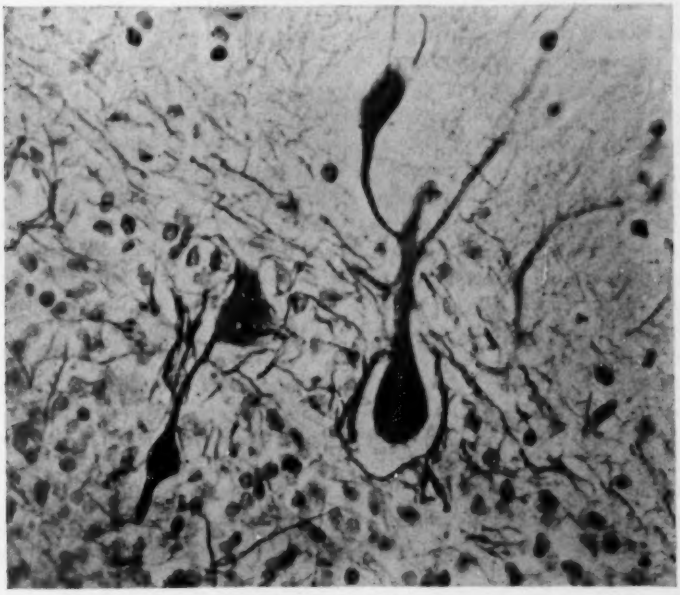

Fig. 6.—Two distorted Purkinje cells showing axonal torpedo (left) and circumscribed dendritic swelling (right); Bodian's strong protein silver impregnation; medium power magnification.

drites of Purkinje cells were irregularly arranged and distorted by circumscribed swellings. Their axons had very often a globoid enlargement (the so-called torpedo) showing strong affinity for silver (fig. 6). In some folia these "torpedoes" were numerous. Occasionally a torpedo was formed by a dark central nucleus surrounded by a large pale halo. Fat stain failed to reveal any fat in the altered cells or in the dendritic swellings. The granular layer was less cellular than in normal controls, and the central core of the folia stained paler than normal in myelin preparations. Cerebellar nuclei showed normal amounts of neurons, but many were shrunken and had pyknotic nuclei. Considerable shrinkage was also noted in the cells of the bulbar olives.

COMMENT

The clinical manifestations of the case reported here leave little doubt as to the diagnosis of Albright's disease. The association of disseminated bone lesions, more pronounced on one side, cutaneous pigmentation and a goiter nontoxic in nature is characteristic of the disease. The finding of normal values for blood calcium and phosphorus confirms the diagnosis.

Pathological examination showed that the osseous lesions were those characteristic of polyostotic fibrous dysplasia, consisting of destruction and connective tissue replacement of the bone. Numerous osteoblasts were present, but no osteoclasts were seen. Foamy cells such as those seen in xanthomas of bone were absent, and in no place was the replacing connective tissue arranged in whorls, as seen in neurofibromatosis. Moreover, no neurofibromas were found.

Pathological changes of the endocrine glands were limited to the thyroid, which presented a nontoxic colloid goiter. Several other cases with enlargment of the thyroid have been reported in the literature,[3] but the significance of these thyroid changes remains unexplained. Some form of hormonal unbalance has been considered as the main factor in the causation of the disease, and the finding of an enlarged thyroid together with precocious puberty in girls has been brought forward as evidence in favor of this hypothesis. Sternberg and Joseph[1] found in their case conspicuous pathological changes of the endocrine glands. The pituitary presented microscopic basophilic adenomas, and there were generalized hyperplasia and pleomorphism of basophilic cells. The thyroid was hyperplastic and contained numerous fetal adenomas. The adrenal showed atrophy of the cortex and relative hypertrophy of the medullary tissue. None of these changes was found in the present instance. It is possible, therefore, that the case of Sternberg and Joseph falls into a special category, as suggested by Dockerty and associates.[4] It is interesting to note that colloid goiter was observed in a previous patient.[3c] In any event the study of the present patient adds no significant data in support of an endocrine causation of the disease. As Albright[5] noted, the dissociated phenomena of the disease could hardly fit into any known endocrinological scheme.

The mental defect and the pathological lesions present within the central nervous system are worthy of brief comment. The convulsions that suddenly began to occur at 5 months of age suggest an encephalitic episode, but the pathological examination showed no sequelae of inflammatory processes. The presence of giant distorted neuron cells, the disorganization of the cerebral cytoarchitecture and the peculiar cerebellar lesions would indicate the presence of a pathological process which may be classified under the somewhat vague terms of congenital development defect or endogenous degeneration.

3. (*a*) Gaupp, V.: Monatsschr. f. Kinderh. **53**:312, 1932. (*b*) Lange, K.: Zentralbl. f. Chir. **65**:2368, 1948. (*c*) Musser, H. H., and Barnwell, R.: Endocrinology **22**:420, 1948. (*d*) Mondor, H.; Ducroquet, R.; Leger, R., and Laurence, G.: J. chir. **53**:593, 1939. (*e*) Falconer, M. A., and Cope, C. L.: Quart. J. Med. **11**:121, 1942. (*f*) Sternberg and Joseph.[1]

4. Dockerty, M. B.; Ghormley, R. K.; Kennedy, R. L. J., and Pugh, D. G.: Albright's Syndrome (Polyostotic Fibrous Dysplasia with Cutaneous Pigmentation in Both Sexes and Gonadal Dsyfunction in Females), Arch. Int. Med. **75**:357 (June) 1945.

5. Albright, F.: J. Clin. Endocrinol. **7**:307, 1947.

It is significant to note that previous cases of Albright's disease with mental retardation have been reported. Thannhauser,[6] and independently Albright and associates,[7] described a 10 year old girl who was late in walking and talking and never attended school. Histological observations of the brain were not published, although it is stated that there was an abnormality in the area of the third ventricle consisting of an accessory subthalamic nucleus. The patient observed by Snapper and Parisel,[8] a girl 10 years of age, is described as mentally defective. Coleman [2] reported that his patient was "definitely dull" with a "childish mental outlook." In other cases there is a possibility of some degree of mental defect. For instance, the patient of Hackett and Christopherson [9] was "very nervous and quite bashful"; Goldhamer's [10] patient was "nervous and unattentive"; the patient described by Mondor and associates [3d] displayed "lack of mental alertness." It seems reasonable to assume, therefore, that mental deficiency is a possible, although not frequent, manifestation of this protean disease.

In considering the controversial problem of the etiology and the nosological classification of Albright's disease, the lesions observed in the central nervous system may be of significance. Two other conditions, neurofibromatosis and tuberous sclerosis, are characterized by a curious combination of skin lesions, bone changes and central nervous system involvement.

In neurofibromatosis skin pigmentations and bone lesions somewhat resembling those seen in Albright's disease are found, and mental defect is observed in about one third of the cases. Within the central nervous system, giant atypical cells of the type here described have been observed.[11] The presence of neurofibromas is a distinctive feature of Recklinghausen's disease, which differentiates it from Albright's disease, but striking similarities exist between the two conditions, a fact already extensively discussed by Thannhauser.[6]

In tuberous sclerosis, mental deficiency and cutaneous lesions are outstanding. The latter include pigmented areas. Osseous changes of the fibrous type have been repeatedly reported.[12] Giant atypical nerve cells of the type described in the present patient are a common and characteristic finding in tuberous sclerosis, in addition to the well known large glia tuberosities of the cerebral cortex.[13] The latter are distinctive manifestations of the conditions which are not observed in Albright's or in Recklinghausen's disease. A tendency toward tumor formation is seen in both tuberous sclerosis and neurofibromatosis but not in Albright's disease.

Apart from a common involvement of cutaneous, osseous and nervous systems, these three distinct conditions possess the common characteristic of a striking variability in the degree to which each system is affected, resulting in frequent

6. Thannhauser, S. J.: Medicine **23**:105, 1944.

7. Albright, F.; Butler, A. M.; Hampton, A. O., and Smith, P.: New England J. Med. **216**:727, 1937.

8. Snapper, I., and Parisel, C.: Quart. J. Med. **2**:407, 1933.

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11. Bielschowsky, M., and Rose, M.: J. Psychol. u. Neurol. **35**:42, 1927.

12. Gottlieb, J. S., and Lavine, G. R.: Tuberous Sclerosis with Unusual Lesions of Bones, Arch. Neurol. & Psychiat. **33**:379 (Feb.) 1935. Kveim, A.: Acta dermat.-venereol. **18**:637, 1937. Henlein, H.: Radiology **35**:701, 1940. Berg, G., and Zachrisson, C. G.: Acta radiol. **22**:425, 1941. Ackerman, A. J.: Am. J. Roentgenol. **51**:315, 1944.

13. Critchley, M., and Earl, C.: Brain **55**:311, 1932.

occurrence of incomplete or even monosymptomatic forms. Thus, in Albright's disease bone changes may occur alone or associated with cutaneous lesions but involvement of the central nervous system is uncommon. In tuberous sclerosis brain lesions alone or associated with cutaneous changes are the rule and bone alterations the exception. In Recklinghausen's disease (neurofibromatosis) cutaneous manifestations are outstanding, cerebral and osseous changes less common.

These similarities among the three conditions suggest that similar pathogenic factors are operating in the three diseases. There is much evidence in favor of the hypothesis that both tuberous sclerosis and neurofibromatosis are an endogenous degenerative condition involving the central nervous system and other organs as well, on the basis of congenital errors of development. That Albright's disease is due to multiple developmental errors has been repeatedly maintained by Albright himself.[14] The fact that in the present case the brain lesions were similar in type to those of tuberous sclerosis and neurofibromatosis adds new evidence for Albright's hypothesis. It appears, in conclusion, that in these three conditions the multiple lesions are the result of complex congenital errors of development involving to varying degrees many types of tissues, mainly the nervous system, the bones and the skin.

SUMMARY

A case of Albright's disease with pathological findings is reported, in which there was clinical and pathological evidence of severe involvement of the central nervous system. The lesions of the central nervous system were somewhat similar to those found in tuberous sclerosis and in Recklinghausen's neurofibromatosis. Some similarities among the three diseases are emphasized, and a common causation on the basis of multiple developmental abnormalities involving various systems is suggested.

14. Albright.[5] Albright and others.[7]

HEMANGIOPERICYTOMA WITH METASTASES

Report of a Case with Autopsy

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LEBANON, PA.

IN 1942, Stout and Murray [1] published a report of nine patients in whom an unusual type of vascular tumor had been found. They suggested the name "hemangiopericytoma" as being properly descriptive of this tumor.

In a previous communication the same authors [2] reported the results of tissue culture studies made on the epithelioid cell seen in glomus tumors. The study was stimulated by a review of the medical literature in which they found that this type of neoplasm had not infrequently been reported occurring in areas of the body where glomuses are not usually found, and they questioned the atypical site of these tumors. They were also concerned with the nature of the epithelioid cell of the glomus tumor and its ability to metastasize.

As the result of their study, they produced evidence that the epithelioid cell of some reported cases of glomus tumors can be identified with the pericyte described in 1923 by Zimmermann.[3] This is a modified smooth muscle cell, with contractile properties, which is wrapped tightly about the capillaries. Since this cell has been found in many parts of the body, an explanation is furnished for so-called glomus tumors occurring where glomuses have never been found.

In the publication reporting nine cases of hemangiopericytoma, Stout and Murray asserted that the histologic pictures were not characteristic of glomus tumors but that they revealed cells typical of pericytes. The tumors were composed of endothelium-lined tubes or solid sprouts surrounded by the rounded cells on a supporting meshwork of reticulin fibers. Some of the rounded cells showed a tendency toward elongation with the appearance of myofibrils. The authors warned against confusing this tumor with endocrine tumors having a rich vascular network.

In seven of the nine original cases reported by Stout and Murray, the growth was localized, wth no evidence of metastasis, although they stated that not all were followed regularly. In one of the cases the neoplasm extended into adjacent tissues,

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Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or the policy of the Veterans Administration.

1. Stout, A. P., and Murray, M. R.: Ann. Surg. **116**:26 (July) 1942.

2. Murray, M. R., and Stout, A. P.: Am. J. Path. **18**:183 (March) 1942.

3. Zimmermann, K. W.: Der feinere Bau der Blutcapillaren, Ztschr. ges. Anat., pt. 1, 1923, vol. 68; Der feinere Bau der Blutcapillaren, Munich, J. F. Bergmann, 1923.

requiring repeated excision before the lesion was cured, and in another case there were metastatic growths in distant organs—the liver, the inguinal lymph nodes and the bones—which appeared four years after the excision of the original tumor of the thigh. A number of other cases [4] have been reported since the original discussion, but in all of them the lesions described were essentially benign. In the cases reported, there was no predilection for specific sites.

REPORT OF CASE

A 39 year old white man was admitted to the Lebanon Veterans Administration Hospital on Dec. 29, 1948, complaining of considerable pain in his right flank. He was having marked hematuria. The history previous to 1940 was not significant, but in that year he noted a soft tissue mass in his left thigh. In 1941 this mass was removed, but to our knowledge no diagnosis was made from the miscroscopic section.

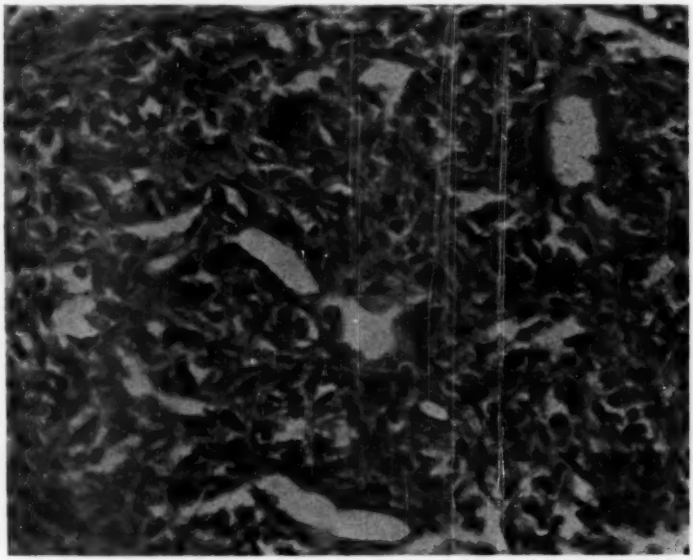

Fig. 1.—Section from the tumor removed from the thigh in 1941; × 280.

In 1944 he was inducted into the Army; after eight months he was discharged because of painful, flat feet. There were no significant symptoms except for occasional nonspecific aches and pains, until in August 1946 when he noticed some pain over the right kidney. He became "jittery" and nervous. In October 1946 he underwent an operation for a right inguinal hernia, and at that time it was noted that he had an enlarged thyroid gland and a lump under the right clavicle. Thyroidectomy was done in December 1946. After a four month convalescence, he returned to work and felt fairly well until August 1947 when he noticed further swelling in his neck. He was admitted to the Bronx Veterans Administration Hospital on Dec. 3, 1947. A biopsy was performed on the right infraclavicular mass. The microscopic sections were compared with those made from the tumor removed from the thigh in 1941 (fig. 1). They were found to be similar, and the diagnosis of

4. Stout, A. P., and Cassel, C.: Surgery **13**:578 (April) 1943. Warren, S.: Tumor Seminar, J. Missouri M. A. **45**:345 (May) 1948. Sims, C. F.; Kirsch, N., and McDonald, R. G.: Hemangiopericytoma, Arch. Dermat. & Syph. **58**:194 (Aug.) 1948.

"malignant hemangiopericytoma" was made. A roentgenogram of the chest, on that hospital admission, revealed shadows which were strongly suggestive of metastases. An intravenous pyelogram revealed distortion and distention of the right renal calyces; roentgenologic study of the upper part of the gastrointestinal tract demonstrated some pressure on the duodenum. At that time he had painful swellings in the fingers, the knees and the ankles. A course of high voltage x-ray therapy was given, and the patient was discharged in March 1948 feeling fairly well, but apparently there was little, if any, diminution in the size of the mass. He started to work in May and had worked 12 weeks when nausea, abdominal pain and swelling of the ankles occurred. The family physician again sent the patient to the Bronx Veterans Administration Hospital, where he was admitted on Aug. 12, 1948. While he was in the hospital, the pain over the right kidney recurred, accompanied by gross hematuria. Nitrogen mustard (HN_2) therapy was suggested, but the patient became homesick and was allowed to leave before the treatment could be carried out. At home he felt fairly well for a few weeks and then had recurring attacks of hematuria. The pain in the right abdominal region became increasingly severe, and the patient grew weaker. Because of the proximity, he was admitted to this hospital. The family physician said that he had treated the patient for three weeks with sodium pteroyltriglutamate (teropterin®).

Physical examination revealed a thin white man with a facies expressing considerable distress. Two fixed, nonpulsating masses were noted in the right lateral aspect of the neck. The scar of the previous thyroidectomy was visible. Over the chest were some areas of decreased breath sounds with exaggerated vocal fremitus. In the abdomen there was a mass the size of a grapefruit to the left of the umbilicus. There was tenderness over the right upper quadrant with a sense of fulness to the examining hand which was thought to be enlargement of the liver. Tenderness was noted over the right renal area, but no masses were palpable. Mild edema was present in the right arm and hand. A scar was observed in the lateral aspect of the left thigh, where the original tumor had been removed. The area in the region of the scar was not indurated. There was bilateral pitting edema of the legs and feet, more marked on the right.

Laboratory Findings.—During the hospital stay, the patient had albuminuria ranging from 1 + to 3 +. The specific gravity of the urine varied from 1.012 to 1.022. Numerous white blood cells were found in all specimens, and in many there were numerous red blood cells. On admission, the hemoglobin content of the blood was 10.4 Gm. per 100 cc., and there were 3,580,000 red cells and 8,200 white cells per cubic millimeter and a normal differential count. The platelets numbered 140,000 per cubic millimeter. The erythrocyte sedimentation rate was 31 mm. per hour. The blood urea nitrogen was 11.2 mg. per 100 cc. The serum calcium was 8.5 mg. and the inorganic phosphorus 2.9 mg. per 100 cc. The Kahn test of the serum was negative. The total proteins were 5.08 Gm. per 100 cc., with an albumin-globulin ratio of 2:1. The carbon dioxide-combining power was 56 volumes per cent.

A roentgenogram of the chest revealed multiple, round, sharply circumscribed, dense shadows in both lungs, varying in size from 8 mm. to 8 cm., and reported as having the appearance of multiple hydatid cysts of the lungs. A flat plate of the abdomen revealed "a large soft tissue mass, presumably kidney," which appeared "to occupy the midportion of the right side of the abdomen." The right psoas muscle shadow was obliterated. An intravenous pyelogram was reported as follows: "The large mass in the right flank is undoubtedly kidney and the urograms depict a typical 'spider leg' deformity of the renal pelvis and calyces on this side. The right ureter did not visualize. The findings are suggestive of hypernephroma involving the right kidney." The left kidney's shadow was normal.

Course in the Hospital.—While in the hospital, this patient required considerable dihydromorphinone hydrochloride U. S. P. (dilaudid® hydrochloride) for relief of the pain in his right lumbar region. Periodically, his temperature rose, at one time as high as 104 F. This elevation returned to normal after two days of penicillin therapy.

As it had been suggested at the Bronx Veterans Administration Hospital that this patient be given a trial of nitrogen mustard (HN_2), it was agreed to administer the drug at this hospital for want of more specific treatment. The patient, whose weight was 146 pounds (66 Kg.), was given 6.4 mg. intravenously daily, beginning February 23. There was a slight amount of nausea after each injection, but the treatment was continued for the full four doses on consecutive days. The mass in the neck, which had been outlined with ink previous to starting the therapy, appeared to decrease in size slightly. There were no other objective changes; the blood picture showed no

untoward response. The patient, however, manifested a rather remarkable change in mental attitude. He stated that he felt very much better and desired to get out of bed, but there was no decrease in the amount of dilaudid® used. A second course of nitrogen mustard (HN_2) was instituted on April 6. At this time the note was made that the patient seemed much more comfortable and stronger than at the beginning of his first course. The dose was increased to 8 mg. daily. He had considerable nausea and vomiting, and the discomfort was so great that after the third daily injection the medication was discontinued at his request. No depression of any of the peripheral blood elements followed this course of therapy. The patient's course was downhill from this time on. He had had a number of episodes of hematuria during his hospital stay, and these increased in severity. The swelling in the neck became greater, and he experienced increasing difficulty in swallowing. Roentgenograms of the chest showed increase in the size of the lesions in the lungs. The lumbar pain required more narcotics for relief, and at times the patient became mentally confused. He died on May 3, 1949. Clinically his death appeared to be due to a respiratory difficulty, probably asphyxia from pressure on the trachea. Permission for autopsy was granted.

POSTMORTEM EXAMINATION

Gross Findings.—The mass on the right side of the neck measured 12 cm. to 14 cm. by 8 cm. to 10 cm. and extended from the level of the superior surface of the thyroid cartilage to the sternal notch and passed beneath this into the anterior-superior mediastinum. The masses on the left side were more discrete and oval, measuring about 4 cm. to 6 cm., and did not extend down to the sternum. The linear scar on the anterior surface of the left thigh showed no induration, nor was there any evidence of a mass beneath the scar.

In the right pleural cavity there was approximately 300 cc. of bloody fluid, and about half this amount in the left pleural cavity. A large, lobulated mass, measuring 10 by 6 by 10 cm., occupied the anterior superior mediastinum directly over the trachea. It was difficult to pass the finger between the posterior part of the mass and the spinal column. The external surfaces of the lungs were studded with multiple nodules. In the abdomen, the colon was displaced anteriorly and downward by two huge, rounded masses, approximately 12 cm. in diameter. These lay one on each side of the midline retroperitoneally, behind the colon. They could be moved slightly but were somewhat attached to the posterior parietes. The edge of the liver extended down to the level of the anterior superior spine of the ilium on the right and was studded with white, round nodules, measuring up to 2 cm. in diameter. On cut section, neoplastic nodules were found throughout; the organ weighed 3,860 Gm. The right kidney weighed 550 Gm., and a large portion of the renal tissue was replaced by white nodules, varying in size up to 6 cm. in diameter. The pelvis of this kidney was dilated, and the ureter was thickened down to the junction of the bladder wall. The left kidney weighed 250 Gm. and contained only a single neoplastic nodule, buried in the cortex. Neoplastic masses were also found in the lung, the pancreas and the adrenal glands.

The tissues about the area of the original incision and the femur beneath the scar were searched for evidence of neoplastic invasion, but none was found.

Microscopic Description.—Sections (fig. 2) of lung, liver, pancreas, retroperitoneal tissue, kidneys, adrenals, subcutaneous tissue and soft tissues near the thyroid gland all showed a similar picture of neoplastic invasion. The neoplasm was characterized by its appearance of small, sharply localized masses made up of a cellular "parenchyma" of neoplastic cells supported on an extremely delicate stroma, which was almost entirely made up of small capillary vessels. The cells had a tendency to

line up on the membrane of the capillary vessel but external to it. Many of them were elongated, some were tadpole-shaped, a few were multinucleated, and many were angular in character; few were rounded. Mitotic figures were not frequent but were found scattered throughout. In some areas the supporting tissue showed such wide anastomoses between capillaries that small alveoli were formed, lined by neoplastic cells. The masses appeared to be growing by compression of surrounding tissue, and none showed hemorrhage or degenerative change. The kidney alone showed some variation from this picture in presenting small, tubule-

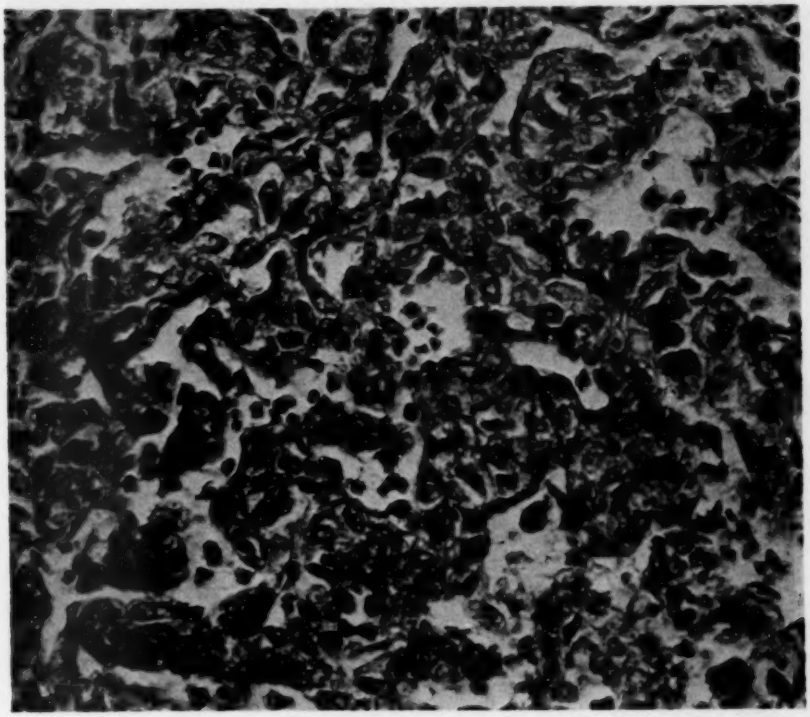

Fig. 2.—Section of a metastasis removed from the lung at autopsy in 1949; × 497.

like structures lined by epithelial cells that were fairly normal-appearing but were in direct contiguity with the neoplasm and had no connection with nearby renal parenchyma. The neoplasm involved both cortical and medullary portions.

COMMENT

As we had had no experience of this type of tumor, we asked Dr. Arthur P. Stout to examine our autopsy sections. At his request, we sent him some tissue-bearing slides prepared with silver reticulin stain. In his opinion, the most successful impreg-

nation with the stain was in the metastatic tumor of the lung (fig. 3), which showed a "very characteristic vascular pattern" of hemangiopericytoma "with many primary vessels from which sprout smaller, secondary ones around which the tumor cells are clustered." The cells were, in most cases, surrounded by a delicate reticulin sheath. By finding the tumor cells outside the vascular sheath, one could be fairly certain that they were not endothelioblasts. The history and the autopsy findings were considered adequate evidence that this was not a vascular endocrine tumor, which sometimes produces a similar pattern.

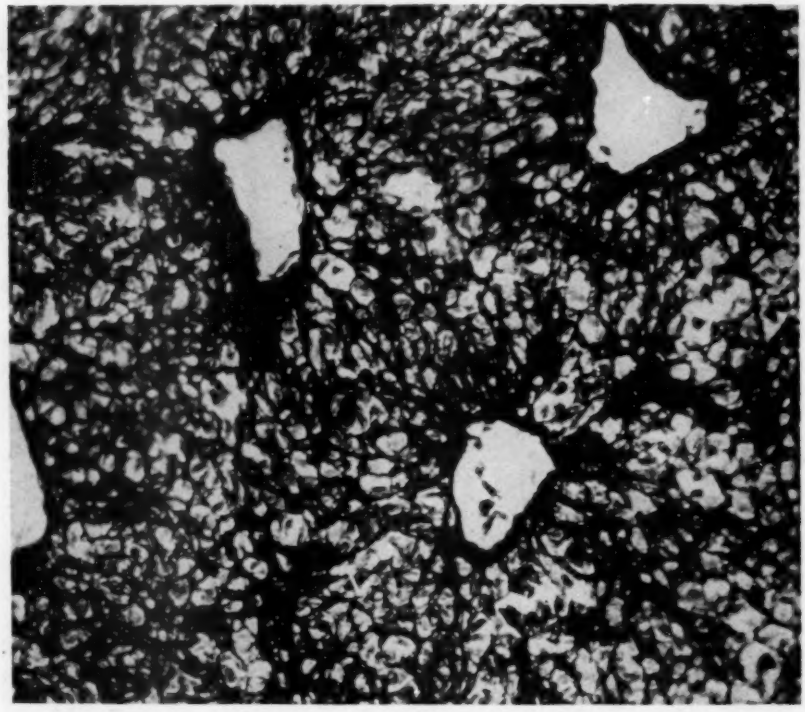

Fig. 3.—Section of a pulmonary metastasis prepared with silver reticulin stain; × 255.

To our knowledge, only one case has previously been reported in which there was metastatic spread. The lesion has been considered essentially benign. Since the above cited series of cases was reported by Stout and Murray, Stout [5] has collected a series in which there was metastasis in 5 cases, but he states that in none was it as widespread as in ours.

There is a striking parallelism in the course of the original case of metastasis reported by Stout and Murray and in our case reported above. In both cases, the primary tumor was removed from the thigh; in theirs, the tumor was attached to the

5. Stout, A. P.: Personal communication to the authors.

femur, while we have no edivence that it was in ours. In neither case was there local recurrence; in their case distant lesions occurred in four years, in our case such lesions occurred in five years.

These cases suggest the possibility that this tumor of pericytes may not be as benign as has been assumed. Maybe, as more late follow-ups are carried out, it will be found that metastasis is not unusual.

We found no reports in the literature to suggest favorable response with the use of nitrogen mustard (HN_2). X-ray therapy had been given with little, if any, improvement on a previous hospitalization and, as the disease was apparently progressing to a fatal outcome, we offered the treatment to the patient on an experimental basis. We had no reason to assume that nitrogen mustard (HN_2) changed the course of the disease. After the first course of the drug, the improvement, objectively, was equivocal, and the subjective improvement may well have been psychogenic. After the second course the patient became progressively worse until the time of his death.

SUMMARY

We have reported a case, with autopsy observations, of an unusual vascular tumor with multiple metastatic lesions. After careful evaluation, it is concluded that this tumor fulfils the criteria necessary for the diagnosis of hemangiopericytoma.[6] The case is unique not only in the rarity of the tumor itself but also in the facts that (*a*) there was widespread metastasis of a relatively benign tumor and (*b*) the metastatic growths appeared five years after the original tumor had been removed and there was no recurrence at the original site.

In this case of hemangiopericytoma, high voltage x-rays and later nitrogen mustard (HN_2) had little apparent effect on the course of the lesions.

6. Stout and Murray.[1] Murray and Stout.[2]

DUPLICATION OF THE MITRAL VALVE

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AND
FREDERICK KELLOGG, M.D.
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DUPLICATION of the mitral valve is an unusual congenital cardiac anomaly. The case presented here is of particular interest because it is the third of 13 instances reported in the medical literature that demonstrates two mitral orifices of equal size, each with a normal set of cusps and chordae tendineae.[1]

REPORT OF CASE

History and Physical Examination.—C. T., a 60 year old white man, was in good health until the onset of angina pectoris in 1937. In 1942 he had an anterior myocardial infarction, and when first seen by one of us (F. K.) in June 1946, he was in mild congestive heart failure. His blood pressure was 110 systolic and 80 diastolic. A faint blowing systolic murmur was heard at the apex of the heart, radiating to the pulmonic area. No diastolic murmur was noted. Fluoroscopic examination revealed moderate enlargement of the left ventricle. The left auricle appeared to be of normal size. Three electrocardiograms, two taken in 1946 and one in 1948, showed evidence of an old anterior myocardial infarction.

Progress.—The patient did well on conventional cardiac therapy. Repeated examinations of his heart revealed no change in the auscultatory and fluoroscopic findings. On Feb. 20, 1950, after two weeks of frequent pain in the chest, he died suddenly.

Autopsy (12 hours after death).—Evidence of an old anterior myocardial infarction was found, and there was a recent occlusion of the circumflex branch of the coronary artery.

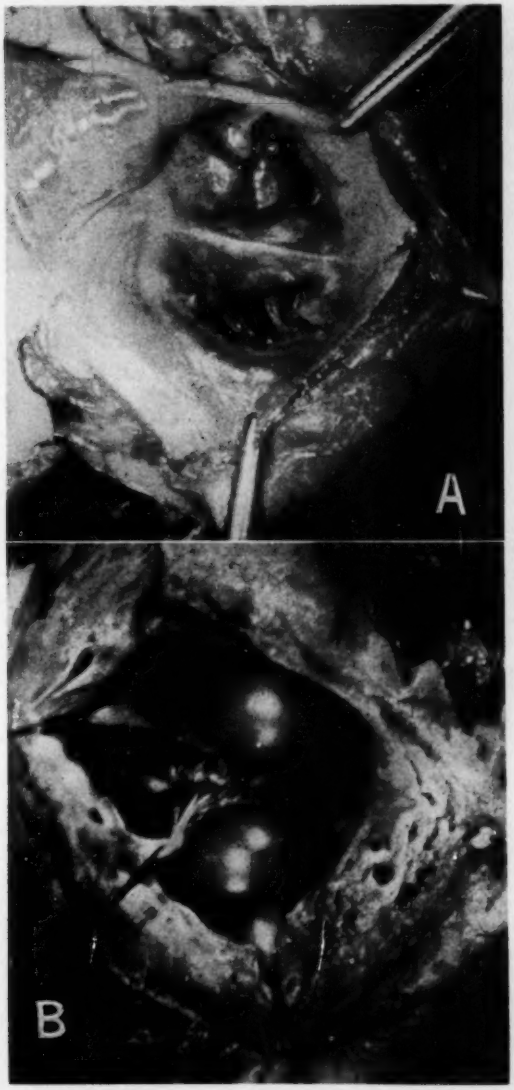
When the left auricle, which was of normal size, was opened, two mitral orifices of equal size were revealed. They were divided by an unscarred transverse bridge 20 mm. long and 10 mm. wide. Each orifice was 20 mm. in diameter and 60 mm. in circumference (*A* in figure).

Each mitral orifice had a pair of normally formed, smooth cusps. Each set of cusps was equipped with a pair of long, slender chordae tendineae. There were four papillary muscle groups, consisting of an anterior and a posterior muscle for each set of cusps (*B* in figure).

No associated developmental anomalies were found.

Dr. Rubsamen is presently Research Fellow in Clinical Biochemistry, Hektoen Institute for Medical Research of the Cook County Hospital.

1. Stuhlweissenburg, O.: Zentralbl. allg. Path. **23**:342, 1912. Camisa, G.: Ibid. **23**:1027, 1912.



A, view of the mitral valves from above showing the transverse band dividing the two orifices.

B, view of the mitral valves from below. The round ends of the demonstrator's fingers are seen holding the transverse band. Threads are attached to the two pairs of chordae tendineae.

COMMENT

In a summary of the previously reported cases of mitral duplication, Schraft and Lisa [2] pointed out that usually the accessory mitral orifice is small and most often lies in the anterior mitral leaflet. Cusps at varying stages of development are present and are usually joined to an anterior papillary muscle. In all instances duplication of the mitral valve was an incidental finding at autopsy.

629 South Wood Street, Chicago 12 (Dr. Rubsamen).

117 East Eighth Street, Long Beach, Calif. (Dr. Kellogg).

2. Schraft, W. C., Jr., and Lisa, J. R.: Am. Heart J. **39**:136, 1950.

SYPHILITIC ANEURYSM OF THE LEFT CORONARY ARTERY

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A SYPHILITIC aneurysm of the descending branch of the left coronary artery is described, which became occluded by thrombus and in this manner produced massive myocardial infarction. Aneurysm of a coronary artery is rare. Scott [1] in a comprehensive and critical review of the literature found 47 cases reported prior to 1948, and I have found one additional case, reported by Ott [2] in 1947. The older literature was extensively reviewed by Packard and Wechsler [3] in 1929, but some of the reports lack detailed information, particularly in regard to cause and to the relation of the lesion to the patient's death.

REPORT OF CASE

An 81 year old Negro man was admitted to the hospital on Nov. 14, 1949, complaining of left lateral chest pain, dysuria, frequency of urination, and nocturia.

He stated that he had not been well for the past three years and that his illness was characterized by generalized weakness and loss of weight. One week before admission he noted pain in the anterior portion of his chest. The exact nature of this pain could not be determined, but it was thought to radiate to the back and down the side of his chest and to have lasted about one day. The patient apparently fainted and was brought to the outpatient clinic. An electrocardiogram revealed an extensive myocardial infarction, and the patient was admitted to the hospital.

He was an emaciated, dehydrated, aged Negro, somewhat lethargic, who complained of weakness. The temperature was 98.0 F.; the pulse rate, 86; and the respiration rate, 18 per minute. The blood pressure was 84 systolic and 60 diastolic. The anteroposterior diameter of the chest was increased, and the percussion sounds were hyperresonant. The lungs were clear. The point of maximum impulse of the heart was feebly felt 7 cm. to the left of the midsternal line in the fifth intercostal space. The left border of cardiac dulness was not outlined because of the increased resonance of the chest. The heart sounds were distant and poorly heard, but there were no murmurs or friction rubs. There was a nodal sinus rhythm.

The salient laboratory findings were as follows: erythrocyte count, 4,730,000 per cubic millimeter; hemoglobin content, 13.0 Gm. per 100 cc.; leukocyte count, 15,850 per cubic millimeter; erythrocytic sedimentation rate, as much as 128 mm. in one hour (Westergren); quantitative Kahn test, 64 units; nonprotein nitrogen content of the blood, 156 mg. per 100 cc.; total plasma proteins, 6.4 Gm. per 100 cc. The urine was acid, gave a 1 + reaction for albumin, and revealed 8 to 10 white blood cells per high power microscopic field. The spinal fluid determinations, including the Wassermann and mastic tests, gave negative results. A chest roentgenogram showed enlargement of the left ventricle and tortuosity of the aorta. Serial electrocardiograms were consistent with the development of an acute anterior myocardial infarction.

From the Departments of Pathology, Emory University School of Medicine and Grady Memorial Hospital.

1. Scott, K. H.: Am. Heart J. **36**:403, 1948.

2. Ott, A.: Wien. klin. Wchnschr. **59**:718, 1947.

3. Packard, M., and Wechsler, H. G.: Aneurysm of Coronary Arteries, Arch. Int. Med. **43**:1 (Jan.) 1929.

The patient was treated with supportive therapy and penicillin. Electrocardiograms continued to display the evolution of an acute anterior myocardial infarction. The patient at no time complained of pain. He died on the twenty-sixth hospital day with the clinical diagnosis of massive myocardial infarction.

Autopsy (four hours after death).—Two centimeters from the left coronary orifice in the longitudinal sulcus on the anterior surface of the heart was a rounded mass which measured 2.5 by 2 cm. and was 1.5 cm. in diameter. It was yellowish gray, moderately firm in consistency and resilient on light palpation. A probe passed into the left coronary artery entered the center of this mass. At the opposite extremity, the mass communicated with the anterior descending branch of the left coronary artery. Section demonstrated this mass to be a saccular dilatation of the anterior descending branch of the left coronary artery. The proximal opening of

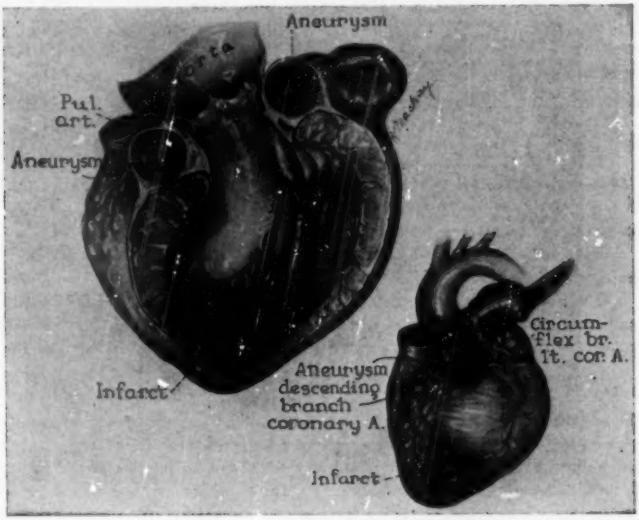

Fig. 1.—Right: Aneurysm of the descending branch of the left coronary artery and area of old and recent infarction involving the anterior aspect of the left ventricle.

Left: Cross section of the aneurysm showing the laminated blood clot and the myocardial infarction involving the anterior wall of the left ventricle and the interventricular septum.

the aneurysm was located 2 cm. from the coronary ostium and approximately 2 mm. distal to the origin of the left circumflex branch of the coronary artery. The outer covering of the aneurysm was yellowish and measured 2 mm. in thickness. The interior was filled by a laminated thrombus. The central portion of this was soft and dark red, while peripherally it was grayish red and distinctly laminated (fig. 1). The distal lumen of the anterior descending branch of the coronary artery was occluded by an extension of the thrombus from the aneurysm.

The coronary orifices were normal in number and location. The left ostium measured 5 mm. in diameter; the right, 6 mm. The proximal 1.5 cm. of the left coronary artery revealed no gross evidence of "tree-barking." The circumflex branch of the left coronary artery was tortuous, had a somewhat thickened wall,

but contained no intimal plaques. The right coronary artery and its posterior descending branch were slightly tortuous, with moderately thickened walls. The intimal surfaces of these vessels were grayish yellow and showed no evidence of atherosclerosis or syphilitic change.

The heart weighed 300 Gm., compared with a normal weight of 306 ± 40 Gm. The wall of the left ventricle measured 1.5 cm. in thickness; that of the right, 3 mm. The valve measurements were as follows: tricuspid, 11.5 cm.; pulmonic, 6.5 cm.; mitral, 10.0 cm.; aortic, 7.2 cm. All valves were essentially normal. The commissures of the aortic valve were not widened, and the entire aorta was smooth, with a yellow intimal lining.

The epicardium was thin and delicate except over the left anterior ventricular surface, where the pericardium was adherent to the epicardium over a considerable extent of the left anterior ventricular area by thin grayish fibrous tissue. In this area the left ventricular wall was pale grayish red, with a central hemorrhagic zone. The anterior left ventricular wall was friable in consistency and retained its shape poorly. The endocardial surface of this wall was rough and covered by a dark red, postmortem thrombus. The underlying myocardium was necrotic. This necrosis extended to the interventricular septum and encompassed the myocardium from the anterior apical segment to the root of the aorta. The area was soft, grayish red, with several central areas of yellowish necrotic myocardium. The endocardium of the auricles and of the right ventricle was thin and delicate. The left ventricle was slightly dilated; the other chambers appeared normal. The foramen ovale and the ductus arteriosus were closed.

Histologic Examination.—The wall of the aneurysm was thin and consisted essentially of hyalinized intima and fibrosed adventitia (fig. 2, upper part). The media had completely disappeared, and only remnants of fragmented elastic fibers could be seen. Some of the vasa vasorum of the adventitia showed extreme narrowing of the lumens resulting from extensive old scarring of the wall. The media and adventitia revealed foci of perivascular cellular infiltrates, consisting chiefly of lymphocytes and plasma cells. A few newly formed capillaries surrounded by similar cellular cuffs extended into the inner layers of the wall (fig. 2, lower part).

The same changes were found in the wall of the left descending branch of the coronary artery immediately distal and proximal to the aneurysmal sac. Here, however, the destruction of the media was less advanced. The adventitia showed distinct fibrosis with old endarteritis of the vasa vasorum and perivascular cuffing of lymphocytes and plasma cells. These cells were also seen about the capillaries extending to the media of the vessel. There was destruction of the elastic tissue but no evidence of arteriosclerotic change. Silver impregnation studies for spirochetes were not done, since histologically the lesion appeared quite inactive.

The thrombus filling the aneurysm was organized in its peripheral portions, but the central areas were more recent and laminated. In the distal portion of the descending branch of the left coronary artery the thrombus was also laminated and recent in appearance.

The sections of myocardium of the left ventricle and interventricular septum showed extensive areas of recent necrosis with hemorrhage and leukocytic infiltra-

tions. In addition, areas of scarring of varying ages were encountered. Some consisted of dense hyalinized collagenous tissue and others of well vascularized granulation tissue with hemosiderin deposits.

Sections of all valves revealed nothing of note.

The descending branch of the left coronary artery, below the aneurysm, had a patent arterial lumen. The media and adventitia were slightly thickened by

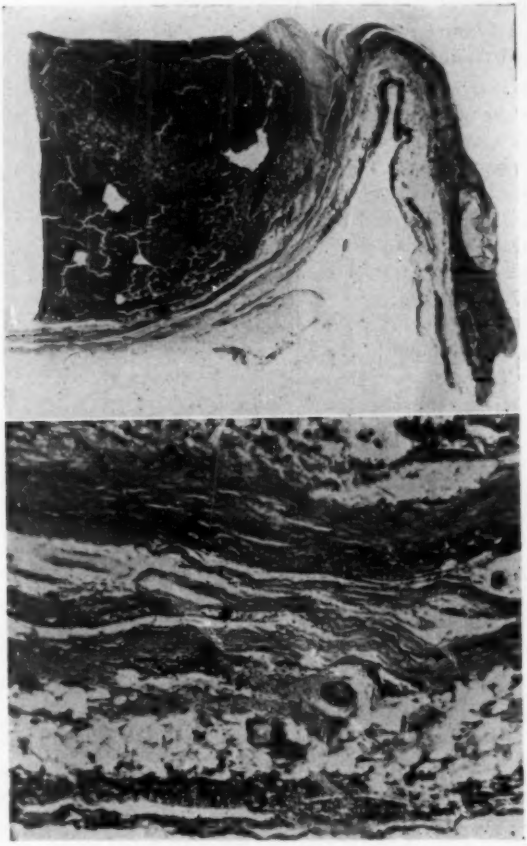

Fig. 2.—Upper part: Low power photograph of a histologic preparation including the aorta (right lower corner), the proximal segment of the left descending coronary artery and part of the aneurysm filled with laminated blood clot. Hematoxylin-eosin; × 7.

Lower part: Wall of the aneurysm showing destruction of the media and fibrosis of the adventitia. The lumen of one of the small vasa vasorum of the adventitia (lower right center) is narrowed by healed endarteritis. Hematoxylin-eosin; × 27.

increased amounts of fibrous tissue. There was no evidence of atherosclerotic change or syphilitic involvement of this segment of the vessel. Sections of the left circumflex and right coronary arteries revealed nothing of note.

The aortic intima was thickened and hyalinized and contained occasional small aggregates of vacuolated large mononuclear cells. The vasa vasorum of the advantitia showed scarring of the media and were eccentrically thickened by a proliferation of fibrous tissue. There was no marked cellular infiltrate about these vessels. The adventitia revealed old fibrosis, but the media was not significantly altered.

Histologic examination confirmed the gross impression of prostatic hyperplasia and emphysema of the lungs.

Anatomic Diagnosis.—Syphilitic aneurysm of the descending branch of the left coronary artery with thrombosis of the aneurysm and of a portion of the descending branch distal to the aneurysm; syphilitic aortitis; old and recent myocardial infarction; focal pericardial fibrous adhesions; hyperplasia of the prostate; pulmonary emphysema.

COMMENT

The findings in our case are those of a syphilitic aneurysm of the descending branch of the left coronary artery. Moritz [4] demonstrated the occurrence of syphilitic coronary arteritis which was not limited to the segment of the vessel in the aortic wall and was sometimes found independent of aortic valvulitis. In none of his cases, however, did the syphilitic arteritis extend beyond the first 10 to 12 mm. of the vessel.

Syphilis is the least common cause of aneurysm of the coronary artery, this being the seventh case reported in the literature. Aneurysm of the coronary artery is most commonly produced by arteriosclerosis but mycotic-embolic and congenital aneurysms have also been encountered. The latter are apt to be multiple. The lesions produced by periarteritis nodosa are not considered in this discussion.

Our patient died from myocardial infarction secondary to complete thrombosis of the aneurysm. Myocardial infarction was rarely noted in the older cases, but Scott [1] cited nine such instances. Rupture of the aneurysm has been reported in about 50 per cent of the older cases, while in others death was attributed to acute or subacute bacterial endocarditis or to rheumatic heart disease with valvular involvement and congestive failure. In some instances the aneurysm was only an incidental finding.

The clinical diagnosis of aneurysm of a coronary artery appears to have been made for only one patient, reported by Ott,[2] in whom, on fluoroscopic examination, a pulsating bulge was noted in the region of the atrioventricular groove. The correct roentgenologic diagnosis was based on the observation that the pulsations of the bulge were not synchronous with those of the ventricle.

SUMMARY

A localized syphilitic aneurysm of the descending branch of the left coronary artery is described in a patient who died of myocardial infarction following complete thrombosis of the aneurysm. In addition, a report in the foreign literature is cited in which roentgenologic study appears to have permitted, for the first time, the antemortem diagnosis of aneurysm of a coronary artery.

4. Moritz, A. R.: Syphilitic Coronary Arteritis, Arch. Path. **11**:44 (Jan.) 1931.

Laboratory Methods and Technical Notes

HISTOLOGICAL DEMONSTRATION OF LIPIDS IN TISSUE AFTER DEHYDRATION AND EMBEDDING IN A POLYETHYLENE GLYCOL

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Studies of arteriosclerosis[1] led us to explore methods for histological demonstration of lipids that might prove more satisfactory than the standard procedures involving frozen sections. The method outlined here has been found useful. A single water-soluble substance is employed for both dehydration and embedding of formaldehyde-fixed tissues. The substance is one of a large class of compounds known as polyethylene glycols or carbowaxes.® Members of this group of compounds are generally designated by their molecular weight. Chemically, the polyethylene glycols (carbowaxes®) are long-chain polymers which polymerize in a compound ether fashion in a straight chain and possess the general empirical formula $HOCH_2$ (CH_2OCH_2) CH_2OH. Their solubility in water (as well as in other solvents), vapor pressure, hygroscopicity and toxicity decrease with the increasing average molecular weight. Their viscosity, inertness and stability increase with the increasing average molecular weight (i. e., carbowaxes® 1000 M.W., 4000 M.W. and 6000 M.W. are solid waxlike compounds, heat stable and chemically inert; carbowaxes® 100 to 600 M.W. are liquids, slightly toxic and chemically fairly reactive). In general the polyethylene glycols are stable to heat and hydrolysis, do not decompose and will not support mold growth. They dissolve in water to form clear, colorless solutions and are soluble in other organic solvents, i. e., ethylene glycols, diethylene glycols, nitroethane, nitromethane, cellosolve, butylcellosolve and, to a lesser extent, in acetone and ethanol. However, we have found that they were insoluble in ether, oils and fats such as olive oil, liquid petrolatum, castor oil, oil of cedar wood at room temperature and animal fats and paraffins at the melting points of the latter two. The chief use of polyethylene glycols has been limited to preparations of water-soluble dermatological ointments and other pharmaceutical preparations.[2] The insolubility of lipids, cholesterol and cholesterol derivatives in the polyethylene glycols at the latter's melting points has made possible the employment of these waxes in dehydration and embedding of tissues in which the demonstration of such substances is desired.

Among those who have employed polyethylene glycols in histological technic are Richards, Anderson and Hance,[3] Steedman,[4] Carsten[5] and Blank.[6] Richards and associates used carbowax® 4000 in preparation of muscle tissue for electron microscopy. Steedman formulated an embedding agent (replacing paraffin) with which the tissue was infiltrated and embedded after dehydration in alcohol. This embedding agent contained diethylene glycol distearate (a carbowax® ester). Carsten employed the polyethylene glycols in conjunction with the distearate in dehydrating and embedding eyes. Blank used a mixture of 9 parts of carbowax® 4000 and 1 part of carbowax® 1500 for dehydrating and embedding tissue sections for routine stains.

Initially we tried various ratios of carbowax® 4000 and carbowax® 1500. Numerous drawbacks were encountered in the method as presented by Blank. The addition of glycerin as an

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1. Rinehart, J. F., and Greenberg, L. D.: Am. J. Path. **25**:481, 1949; Pathogenesis of Experimental Arteriosclerosis in Pyridoxine Deficiency, A. M. A. Arch. Path. **51**:12 (Jan.) 1951.

2. McClelland, C. P., and Bateman, R. L.: J. Am. Pharm. A. **10**:30, 1949.

3. Richards, A. G., Jr.; Anderson, T. F., and Hance, R. T.: Proc. Soc. Exper. Biol. & Med. **51**:148, 1942.

4. Steedman, H. F.: Nature, London **156**:121, 1945.

5. Carsten, M. E.: Rapid Procedure for Embedding of the Eyes, Arch. Path. **44**:96 (July) 1947.

6. Blank, H.: J. Invest. Dermat. **12**:95, 1949.

emulsifying and softening agent proved helpful. However, owing to the large size of the molecules in carbowax® 4000, infiltration was slow and dehydration and hardening of the tissue sometimes occurred before thorough infiltration. Delicate tissues tended to macerate and quite often disintegrate when floated. Another drawback to the use of this mixture is that it did not adequately penetrate and infiltrate fat-laden or adipose tissues.

Carbowax® 1000 was found to be a satisfactory substance both as a dehydrating and as an embedding agent. Unlike carbowax® 4000, it is uniform and not brittle or granular. It also differs from the so-called carbowax® 1500 in being a single compound, while the latter is a blend of solid and liquid polyethylene glycols. The infiltration of carbowax® 1000[7] into tissue is remarkably fast without causing significant shrinkage or hardening of the tissue. Tissues with a high lipid content are readily infiltrated, as are bloody tissues, such as the spleen.

METHODS

Dehydration and Embedding.—Prepare stock solutions or carbowax® 1000 by heating the wax to a relatively high temperature until vapor appears on the surface of the melted wax. Keep in an oven adjusted to a temperature of 48-52 C. Seventy per cent and 90 per cent solutions are made by addition of water and kept in the oven for use.

After formaldehyde fixation, appropriate blocks of tissue are trimmed to 3 or 4 mm. in thickness. With blood vessels it is wise to remove excess fat from the adventitia to avoid spillover of fat in staining. Place the tissue in 70 per cent carbowax® solution in the oven for 30 minutes. Occasional stirring will speed dehydration and infiltration. Transfer the tissues to 90 per cent carbowax® solution for 45 minutes and occasionally stir and rotate the sections in the wax (fatty tissue usually floats on the surface of the wax) solution, causing the dehydration of the immersed portion while the portion above the surface is not dehydrated. Therefore fatty tissues should be fully immersed in the wax. For this purpose one can tie the tissue with a string to a small metal weight anchored at the bottom of the container, thus suspending the tissue in the wax, or the sections may be placed in tissue containers. Transfer the sections to undiluted carbowax® 1000 for one hour, occasionally rotating and stirring the tissue in the wax. Finally embed the sections in carbowax® 1000 in paper cups (do not use metal cups) and place immediately in the icebox to harden. Care should be taken not to allow water to come in contact with the block. Everything with which the block may come in contact should be dry (i. e., microtome knife, forceps, fingers and brushes).

When the wax hardens, trim the blocks with a heated paraffin knife, using a gentle sawing motion without employing too much pressure. Attach the block with heat to a wooden or fiber holder, and put the blocks in the refrigerator for 15 to 30 minutes before cutting.

Sectioning.—Cutting is done as with paraffin blocks. Sections can be cut as thin as 2 microns. Handling the surface of the block with the hand during the process of cutting should be avoided; it causes the surface to be unctuous and prevents ribbon formation in cutting. A clean dry camel's hair brush is useful in handling sections.

Float sections on a solution of the following composition:

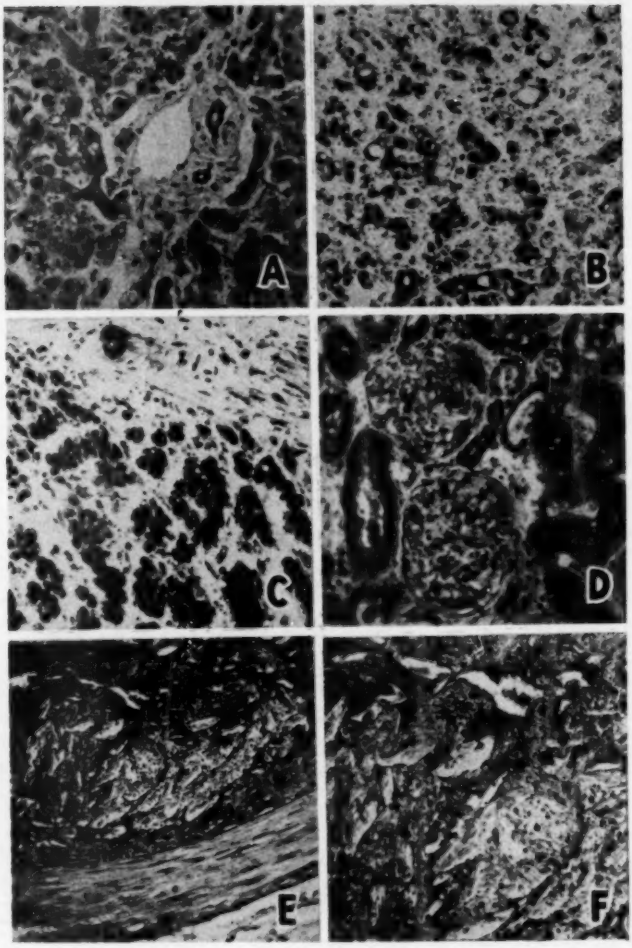
Diethylene glycol	40 parts
Water, distilled	50 parts
Solution of formaldehyde (stock 40%)	10 parts

This is used at room temperature. The glycol protects the delicate sections from maceration and shredding such as would occur in water. It also prevents the sections from drying and inhibits coalescence of fatty globules. Gelatin is the affixative used. The composition and preparation are set forth below:

Gelatin (granular)	10 Gm.
Water, distilled	60 cc.
Glycerin	50 cc.
Phenol	1 Gm.

Dissolve gelatin in water with gentle heat (32-35 C.), and when it is completely dissolved, add the glycerin, filter through gauze cloth and add the phenol. Spread a thin film of the gelatin

7. Carbowax® 1000 is manufactured by, and is obtainable from, Carbide & Carbon Chemicals Corporation, 30 East Forty-Second Street, New York 17.



A, portal area of human liver, structure essentially normal. A few fine fat droplets are present in the liver cells. Note histological detail and absence of shrinkage. × 130.

B, severe fatty infiltration of liver with diffuse fibrosis—from a pyridoxine-deficient monkey. × 130.

C, outer segment of adrenal cortex from a case of essential hypertension. Lipid droplets fill the adrenal cortical cells. Note also the lipid in the sclerotic arteriole of the capsule. × 130.

D, extensive fatty degeneration of the tubular epithelium of the kidney from a pyridoxine-deficient monkey. There are also fine fat droplets in the glomerular epithelium. × 130.

E, human coronary artery showing fine and coarse lipid droplets and cholesterol crystals in the depths of an arteriosclerotic plaque. × 130.

F, segment of the same arteriosclerotic plaque shown in *E*. Note extremely fine fat droplets as well as crystals of cholesterol. × 260.

mixture on the slide and pick up the sections on the slide; drain and wipe well. Formaldehyde solution in the floating solution will denature the gelatin and render it insoluble, causing the section to adhere to the slide. Slides are then left to "dry" in the air or are dried for a short period (five to 10 minutes) in a 37 C. oven. Complete drying should be avoided. Slides may be left for four to five days before staining, or as long as some glycol remains, which protects the sections from complete drying. After sectioning the cut surface of the block it may be coated with the wax, which serves to protect the embedded tissue. Blocks are then stored in a closed container in a cool place.

Staining.—A variety of fat stains have been used in this laboratory, among which are oil red O, sudan IV, sudan black B and coccineal red. Sudan IV has proved to be most satisfactory for general use. A saturated solution of any of these dyes in absolute ethyl alcohol serves as a stock solution. Just before use the stock solution is diluted (40 parts of water to 60 parts of stock solution), thoroughly mixed, allowed to stand for eight to 10 minutes and filtered through a coarse filter paper. The staining procedure is as follows:

1. Dip slides in 50 per cent alcohol once or twice and transfer to the stain for five minutes.
2. Dip in 50 per cent alcohol until the section is clear and transfer to distilled water.
3. After wiping the excess fluid from the slide transfer to Harris' hematoxylin for five minutes.
4. Dip gently in distilled water until the hematoxylin ceases to run and dip in 1 per cent aqueous solution of acetic acid.
5. Rinse gently in distilled water and transfer to an aqueous solution of lithium carbonate (half saturated), to blue the hematoxylin. Rinse in distilled water and mount. (If sudan black B is used, replace hematoxylin with 1 per cent solution of Bismarck brown R in 1 per cent aqueous acetic acid and stain for 5 to 10 minutes. Rinse in 1 per cent aqueous acetic acid, followed by distilled water and mount.)

The most satisfactory mounting medium for semipermanent preservation is either Apathy's gum arabic syrup or a gelatin-glucose solution. The latter consists of a mixture of equal parts of saturated solutions of gelatin and of glucose to which a crystal of thymol is added.

COMMENT

Employed as outlined a polyethylene glycol, carbowax® 1000, has proved to be an efficient dehydrating and embedding agent. Formaldehyde-fixed segments of tissue of the usual size taken for paraffin procedures are quickly dehydrated and infiltrated with the carbowax.® On the average this can be effected in three hours. The block is of favorable consistency for cutting thin sections. The sections can be floated without disruption in an aqueous solution containing 40 per cent of diethylene glycol and formaldehyde solution U.S.P. diluted 1:10. The method involves little or no shrinkage of the tissue.

The lipid substances appear to be maintained in the existing state of droplet size and distribution. Even crystals of cholesterol present in some arteriosclerotic blood vessels may be sectioned and maintained *in situ*. Representative sections prepared and stained in the manner described are shown in the accompanying figure. The brilliant red fat droplets stained with sudan IV cannot be adequately reproduced in black and white. It is possible to secure much thinner preparations than can be made by the frozen section method. The method also provides a permanent block available for future reference.

We have been chiefly concerned with the application of the method in histological demonstration of lipids. The inert character of the carbowax® would probably make it a suitable dehydrating and embedding agent for other histochemical techniques. Certain technical difficulties are encountered in applying the standard staining methods involving alcohols and the usual clearing agents to sections prepared in this manner. At present we are endeavoring to overcome these difficulties.

SUMMARY

A rapid and relatively simple histological method has been devised for demonstrating lipids in tissue. By using a polyethylene glycol known as carbowax® M.W. 1000 as a dehydrating and embedding agent, thin sections are readily prepared. Techniques for handling and staining sections are detailed.

News and Notes

Supplementary Edition of Motion Picture Reviews Now Available.—The Committee on Medical Motion Pictures of the American Medical Association has completed the first supplement to the second revised edition of the booklet entitled "Reviews of Medical Motion Pictures." This supplement contains 86 reviews of medical and health films reviewed in *The Journal of the American Medical Association* from Dec. 31, 1949 to Jan. 1, 1951. Each film has been indexed according to subject matter. The purpose of these reviews is to provide a brief description and an evaluation of motion pictures which are available to the medical profession.

Copies of this supplement are available upon request to:

The Committee on Medical Motion Pictures, American Medical Association, 535 North Dearborn Street, Chicago 10, Ill.

Book Reviews

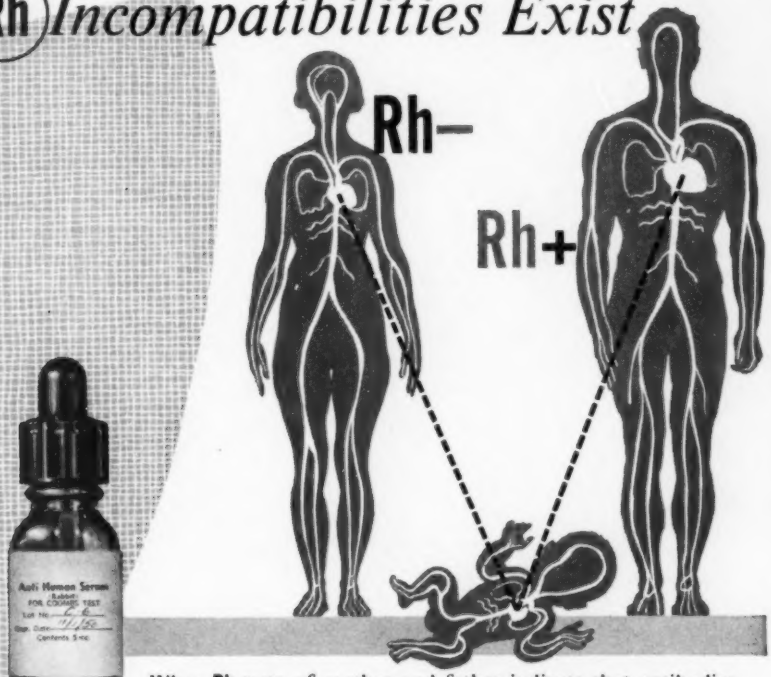
BIJNIERSCHORS EN ZIEKTE: EEN CHEMISCH EN MORPHOLOGISCH ONDERZOEK. By Aart Schaberg. Pp. 132, with 42 illustrations and 14 tables. H. E. Stenfert Kroese's Uitgeversmaatschappij. N.V., Breestraat 14, Leiden, 1951.

DIABETES GUIDE BOOK FOR THE PHYSICIAN. Pp. 79. American Diabetes Association, Inc., New York, 1950.

AN INTRODUCTION TO PATHOLOGY. By G. Payling Wright, D.M., F.R.C.P., professor of pathology, Guy's Hospital Medical School, University of London. Pp. 569, with nearly 250 illustrations, of which more than half are photomicrographs. Price $6. Longmans, Green & Co., 55 5th Ave., New York 3; 215-219 Victoria St., Toronto 1; West End, 6-7 Clifford St., London, W., 1950.

This leisurely and rather philosophical book represents an unusual approach to the teaching of pathology and is almost entirely introductory in function. Its content is that of so-called general pathology and includes no sections on systemic pathology. The material is somewhat unconventionally arranged, but the style and format are clear and the illustrations numerous. Little attempt is made to describe specific diseases or to furnish an outline of diseases, although basic processes and types of lesions are discussed at greater length than in other textbooks.

To Insure the Infant's Health if Rh Incompatibilities Exist



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